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SPOROGENESIS IN NEPHRODIUM

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SHIGÉO YAMANOUCHI

(WITH PLATES I–IV)

Historical review

There have been published during the past three decades a few accounts of sporogenesis in pteridophytes. Since cytological studies have advanced rapidly in recent years, many views which were once prevalent have been proved to be erroneous, or at least to be capable of a different interpretation, and the authors themselves may have at present entirely different views from those which they held when the accounts were published. It seems worth while, therefore, to make a historical review of some of the work hitherto published.

There is first a paper by HUMPHREY (**29**) on the study of the spore mother cells of Osmunda, Psilotum, and other forms, in which the main purpose was to prove the presence of extranuclear centrosomes in plants above the bryophytes. His account of the number of chromosomes in Osmunda was taken up by STRASBURGER (**46**) in his data dealing with the periodic reduction of chromosomes in the life-history of organisms, as one of the instances which prove the constancy of the number of chromosomes in a certain period of the life-cycle.

OSTERHOUT'S (**35**) studies on Equisetum were really one of the most important contributions to our knowledge of the spore mother cells of higher plants. He found that the spindle at the outset is multipolar polyarch, according to the terminology of STRASBURGER (**47**), and that the numerous cones become grouped around the

common axis, but the poles of the spindle at metaphase hold their composite nature with entire absence of the focal center or centrosome. This account, which did much to dispose of the current idea that the development of the spindle in higher plants is controlled by centrosomes, is noteworthy.

Then appeared two papers with detailed description of the method of division and distribution of chromosomes: one by CALKINS (**11**) on *Pteris tremula* and *Adiantum cuneatum;* and the other by STEVENS (**44**) on *Pteris aquilina, Cystopteris fragilis,* and *Scolopendrium vulgare.* CALKINS believed that there was a tetrad formation of chromosomes in the prophase of the spore mother cell of ferns, and that the process of sporogenesis in those forms followed the same course as was observed in the maturation division of sexual cells in animals, with a transverse division and consequently a qualitative reduction in WEISMANN'S sense. STEVENS' studies on the forms named above disagree with CALKINS' view in several particulars, the main point being that there is present no transverse division of chromosomes in the spore mother cell, but two longitudinal divisions, and therefore there is merely a quantitative reduction. STEVENS' observation on the formation of daughter chromosomes in the second division was criticized by STRASBURGER (**47**).

SMITH (**42**) investigated the sporogenesis of *Osmunda regalis.* His results agree with those of OSTERHOUT in regard to the absence of centrosomes, and differ as to the origin and development of the spindle; he held that the spindle was bipolar from the beginning.

In 1904 GREGORY'S paper (**26**) was published. His account covers the sporogenesis of eight genera of ferns, i. e., Pteris, Scolopendrium, Asplenium, Onoclea, Davallia, Fadyenia, and two other forms among Cytheaceae, and seems to confirm FARMER and MOORE'S interpretation (**18**) of the formation of chromosomes at the heterotypic division, indicating the occurrence of a transverse division.

The next year STEVENS (**46**) published his results on the spore formation of *Botrychium virginianum.* His account begins with his so-called early prophase of the spore mother cell; the first appearance of the spindle of the first and second divisions, he finds, is within the nucleus. As regards the heterotypic mitosis, he states that the chromosomes in metaphase separate in the line of transverse fission

and that he agrees with the views maintained by FARMER and MOORE (**18**), GREGORY (**26**), and STRASBURGER (**49**).

CARDIFF'S (**12**) studies of synapsis and reduction in several forms of flowering plants and in *Botrychium obliquum* paid particular attention to the formation of the spirem and its behavior up to the formation of bivalent chromosomes in the heterotypic mitosis. According to him, paired threads, which appear in the presynaptic stage of the nucleus, fuse during synapsis and again split longitudinally after synapsis, probably along the line of the previous fusion.

In 1907 a paper entitled "Studies in apospory and apogamy in ferns" appeared under the joint authorship of FARMER and DIGBY (**16**), whose two preliminary notes were published in 1903 and 1905. This paper deals directly with the matter indicated by the title, but some stages in the division of the spore mother cell are described in three forms of Lastrea and one species of Scolopendrium. The paper is accompanied by many important figures, but only three of the spore mother cells with heterotypic chromosomes are given, so far as sporogenesis is concerned.

Recently there appeared two papers, one by BURLINGAME (**10**) on Ophioglossales, and the other by GRÉGOIRE (**24**), with the title "La formation des gemini hétérotypiques dans les végétaux." BURLINGAME'S paper is chiefly devoted to a study of the sporangium. From the cytological standpoint GRÉGOIRE'S paper is very important. His views, which were published in 1904 (**25**), concerning the structure of the resting nucleus of the spore mother cell, interpretation of synapsis, formation of chromosomes of the heterotypic mitosis, and distribution of the daughter chromosomes in the first and second divisions, were based chiefly upon BERGH'S studies (**6, 7**) of Allium, Lilium, and other forms; but in this recent paper GRÉGOIRE records the results of his own examination of *Osmunda regalis* and four monocotyledons, which confirm his previous views.

Nephrodium

My present investigation of *Nephrodium molle* Desv. was carried on in order to understand the characteristics of the nuclear behavior of this species, and to obtain the knowledge prerequisite for a study of apogamy in the same species. Consequently this paper should

be regarded as introductory to a paper entitled "A study of apogamy," which will appear later. Much attention has been paid to the behavior of chromosomes, combining observations with theoretical views, with a hope of securing an explanation of the phenomena observed, so that the chromatin substance will be the chief object treated in the present paper.

I wish to express my gratitude to Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN for their kind advice and criticism during the progress of this work. I am also under obligation to other members of the botanical staff for courtesies extended to me.

MATERIAL AND METHODS

The material for this investigation was collected in greenhouses of the Hull Botanical Laboratory, and in Lincoln and Washington parks, Chicago, from October 1906 to March 1907.

Killing and fixation were made in various fluids, among which FLEMMING'S weak solution, with a little modification in the percentage of acetic acid, proved most effective. The material was left in the fluid for two to four hours, then transferred into fluid of the same formula, but without osmic acid, ten to twenty hours. The thoroughly washed material was passed very carefully through a series of alcohols beginning at three per cent. and imbedded in 48° to 52° paraffin. The sections were cut 3–5 μ in thickness and stained with safranin gentian-violet, or iron-alum-hematoxylin, sometimes combined with counter-stains, such as safranin, orange G, eosin, or Congo red. A Zeiss apochromatic immersion 1.5^{mm}, N. A. 1.30, and compensating oculars were used for studying the preparations.

DESCRIPTION

Vegetative mitosis in the sporophyte

A leaf too young to produce sori shows in its various parts the successive stages in vegetative mitosis. The form of the mitotic figure is affected by irregularities in the size of the cell which contains it, and the abundance of plastids, oil, and other cell contents interferes somewhat with accurate observation. The mitotic figures which occur during the formation of sporogenous tissue, from the first division of the archesporial cell to the completion of spore mother cell, proved

most favorable, being entirely free from such obstructions, and these mitoses will be used for the following description.

The resting nucleus in the sporogenous tissue contains a chromatin reticulum with one or several nucleoli. The general structure of the nuclear reticulum needs mentioning. It does not seem to consist of two distinct elements, chromatin and linin, with chromatin granules distributed irregularly upon or imbedded in the linin ground-substance, but it seems to be composed entirely of chromatin material. The appearance which indicates that there may exist a mixture of two substances—the dark-stained masses and light-colored strands—does not seem to be due necessarily to a different affinity for stains, but to the fact but that the more compact regions of chromatin material are more affected by stains than the more loosely aggregated regions, which are lightly stained.

The number of nucleoli is variable and there seems no rule for their position in the nuclear cavity, for sometimes they lie very close to the membrane and sometimes they are centrally located. The form is rounded in most cases, but often they are aggregated into one or two irregular masses. Even in the resting nucleus they show a vacuolized structure.

The chromatin reticulum in the resting nucleus is irregularly distributed, often great masses being located in the center or sometimes eccentric near the periphery, leaving the center vacant. Approaching the prophase, there seems to be a tendency of some of the chromatin material to form a continuous spirem, i. e., some fine strands of the chromatin may break apart entirely or may become thicker by getting the material from some ragged chromatin clumps which consequently diminish in size. The spirem, uneven at first, becomes gradually uniform in thickness and distributed throughout the nuclear cavity (*fig. 1*). Whether there is present a single spirem or not was not determined. Close examination of this stage in a number of preparations showed that it was hardly possible to make out free ends, and this may be taken as an evidence that the spirem is a continuous one. Nucleoli persist in positions distinctly isolated from masses of the chromatin reticulum, so that they do not seem to contribute to the establishment of the spirem through the migration of material. Usually at this stage they seem to unite into one mass.

While these events are going on within the nucleus, there are formed kinoplasmic caps at two poles outside. The kinoplasmic cap at first consists of a few fibers arranged in a cone, the fibers evidently running in a longitudinal line, one end focusing at one pole, and the other end diverging toward the nucleus, reaching nearly to the nuclear membrane or falling short of it. Since no change ever occurs in the nuclear membrane, these fibers certainly originate from the rearrangement of the cytoplasm where the cone arises. The whole cytoplasm, except where the kinoplasmic caps arise, shows at this time a fine fibrillar structure. Now the nuclear membrane begins to disappear at the basal region of the kinoplasmic caps, the rest of it still clearly limiting the nuclear contents from the cytoplasm outside. There seemed to be no regularity in the course of the spirem until this stage. The entangled spirem was distributed throughout the cavity, the bent parts of the loops being directed toward the membrane; but when the nuclear membrane begins to disappear near the kinoplasmic caps, some parts of the spirem seem to move out in the direction of the caps.

Then follows the entire disappearance of the nuclear membrane, the segmentation of the spirem into a certain number of slender chromosomes, and the intrusion of spindle fibers which become attached to the chromosomes at their ends (*fig. 2*). At this time there is first observed the longitudinal splitting of the chromosomes, the two parts resulting from the splitting always remaining closely associated. The chromosomes, in general, are directed along the axis of the two poles; one end reaches near the pole, and about the equatorial region lies the other end, to which spindle fibers from the two opposite poles are attached; so that the distance of the attached ends from the poles is approximately the length of two chromosomes. This stage passes into the condition shown in *fig. 3*, where the chromosomes become much less curved. The line associating the two chromosomes which resulted from the longitudinal splitting is still visible. The chromosomes which were so slender and peculiarly curved, as stated above, from the first segmentation to this period, now begin to shorten; the equatorial ends, to which the spindle fibers from the two poles are attached, remain stationary, while the ends directed to the poles move toward the equator as the chromosomes shorten. In the course of

shortening, a stage such as that represented in *fig. 4* has been described by some authors as being a typical figure of vegetative mitosis in ferns.

However, the shortening of the chromosomes proceeds still farther, until the bent ends lying at the equatorial region become straightened, and the chromosomes become rod-shaped; thus there is established the stage in which the chromosomes are arranged in an equatorial plate (*fig. 5*). The two daughter chromosomes formed by the longitudinal splitting of a single one at prophase have remained side by side and have been affected equally by the process of shortening, so that now they are almost similar in size and form. When this stage of the equatorial plate is attained, the two daughter chromosomes seem to begin to separate at once. This stage is less frequently met than the earlier or later stages, even in material favorably fixed and showing an abundance of stages.

The separation of many daughter chromosomes generally begins and proceeds simultaneously, but rather rare instances are observed of several tardy ones, which separate after their associates are already on the way toward the poles. The chromosomes arranged in the equatorial plate are perfectly straight; the separation of the two halves begins at the ends where the spindle fibers are attached and proceeds toward the other end, necessarily causing a curving, as represented in *fig. 6;* but soon after their complete separation they resume their straight form and so persist. When the two sets of daughter chromosomes reach nearly to the poles, they become perfectly parallel, without overlapping (*fig. 8*), and in the more favorable cases they are as regularly placed as if they stood in one plane, a stage which is the most satisfactory for counting. The polar view of this stage (*fig. 9*) shows that the number of chromosomes is 128 or 132. During prophase and even metaphase the number of chromosomes could sometimes be estimated, especially in the polar view at the equatorial plate stage, but not with such exactness as in the early telophase.

The chromosomes grouped at the pole are drawn so tightly together that the ends toward the pole become closer than the ends directed toward the equatorial region. This aggregation process goes on farther and there results an extremely dense mass, in which it is difficult to trace the outlines of each chromosome. The chromosomes

begin to be vacuolized, i. e., chromatin materials which constituted the chromosomes in their compact condition become localized in an irregular way, in some places becoming quite loose, and in others still remaining as a dense aggregation. All the chromosomes undergoing this change are associated, the ultimate result being a vacuolized mass of chromatin material from all the chromosomes (*fig. 10*). By the interaction between the cytoplasm which surrounds these vacuolized masses and nuclear sap which may be secreted within the vacuolized region of the chromosomes, there is formed a nuclear membrane. As the nuclear sap increases in amount, the nucleus grows in size (*fig. 11*).

The polar end of the daughter nucleus at the time of the formation of the membrance is always concave (*fig. 12*), very likely due to the fact that before the vacuolization the face ends of daughter chromosomes pointed to the pole are drawn together toward the pole so as to come nearer, and that in this position vacuolization has proceeded. It seems probable that the vacuolization is more pronounced in that concave region than elsewhere, so that in the young daughter nucleus the chromatin material is rather scanty at the region of the previous pole, where immediately after the organization of the daughter nucleus one or two new nucleoli appear (*fig. 13*). It may turn out that the daughter nucleus thus reconstructed shows polarity.

Coincident with the construction of the daughter nuclei there appear granules in an equatorial plane upon fibers of the central spindle. The granules first make their appearance upon the fibers in the central part of the equatorial plane and then toward the periphery. It seems reasonable that the fiber substance has contributed the material for the formation of the granules.

Contemporary with the completion of the cell wall, growth of the daughter nuclei continues and the chromatin material assumes again a ragged structure on account of further continuation of vacuolization. The general outline of the daughter nucleus becomes nearly spherical and there is established the resting nucleus of the succeeding mitosis (*fig. 14*).

The processes of nuclear division from the reticulum of the resting condition to the reconstruction of the next resting nucleus are carried on as described above in any vegetative mitosis in Nephrodium, and

the sequence of mitosis preceding the formation of the nucleus of the spore mother cell is exactly the same.

Sporogenesis

PRESYNAPTIC STAGE.—The chromatin reticulum in the resting nucleus of the spore mother cell has the same structure as was just described for the nucleus of the sporogenous cell; the size of the nucleus, after its full growth, is greater than that of any nuclei of the sporogenous cells, and not infrequently it occupies almost all of the cell. No regularity could be found in the massing of the chromatin material; at certain points there are dense masses and at others almost none at all. Nucleoli, one to several in number, are always present in various locations, without any physical connection with the chromatin.

The exact structure of the chromatin reticulum needs description. It is an anastomosing complex of ragged clumps and irregularly branched strands (*fig. 15*); the former always stain deeply and the latter lightly, simply because of the difference in compactness of massing. They originated from a single source—the chromosomes of the previous mitosis—by the vacuolization process described above.

The clumps of the reticulum lose gradually their ragged look, because of the disappearance of the fine strands, the elongation of the clumps in certain directions, and the migration of material from the clumps to some of the fine strands so as to thicken them (*fig. 15a*). All these changes tend to transform the ragged structure, but they do not occur simultaneously throughout the reticulum, so the whole is still plainly a reticulate structure, as seen in *fig. 16*. Nevertheless, in regions where the reticulum is being converted into thread structure there are visible parts of threads running closely side by side (*fig. 16a*).

SYNAPSIS.—The transformation of the reticulum into a thread structure now proceeds rapidly. When the transformation is nearly completed (*fig. 17*), parts of the thread are not smooth and uniform in appearance and thickness, and in its course it is greatly tangled. These tangled masses of thread begin to contract and become localized on one side of the nuclear cavity, a process which marks the initiation of the synaptic state. Preparations stained with iron-alum-hematoxylin and extracted to a certain degree of differentiation of the stains,

show that this loosely tangled mass consists of lightly colored threads and deeply stained granules; undoubtedly the thread in this tangled and contracted condition is not homogeneous throughout; on the contrary, there are present not only irregularities in thickness in parts of the thread, but also the chromatin material is more compact in certain parts and more loosely aggregated in other parts.

That the parts of the thread in this stage are double is fairly discernible (*fig. 17a*) in tangential sections of the nucleus; undoubtedly this doubling has come from the changes observed in the earlier stage represented in *fig. 16* as originating from the transformation of the reticulum; therefore this double part of the thread cannot be recognized as an indication of a new longitudinal splitting, but as two threads independent from the start.

These double threads run side by side very closely, as if they were fusing. Whether they are completely fused or not could not be determined, but their association is very close. These masses of threads become more tightly drawn together and contracted into a ball which seems to be the climax of the synaptic stage (*fig. 18*).

FORMATION OF HETEROTYPIC CHROMOSOMES.—The course of the thread or spirem is irregular in the synaptic stage; it is twisted and generally much curled, but there are present several parts of the spirem which run comparatively straight across the contracted ball of the spirem from near the nuclear wall with which it lies in contact. The loops happen to be formed near the periphery of this contracted mass, but they have nothing special to do with the formation of chromosomes.

In the majority of cases the nucleoli seem to unite into one, which becomes caught in the tangled mass in the early synaptic stage, generally far from the nuclear membrane, and its spherical form persists.

The spirem of this stage shows a relatively smooth structure and it is not easy to distinguish its double nature throughout, probably on account of the close association of the two independent parts; but tangential sections reveal that the parts of the spirem are unmistakably composed of two threads running side by side, often diverging rather widely (*fig. 19*). It seems highly probable that we see here an indication of the separation of two threads which were first observed in the presynaptic stage (*figs. 16, 16a*) and entered into synapsis, as

already described. Now the tangled mass of the spirem begins to uncoil (*fig. 20*) slowly; superficial parts of the spirem in the synaptic mass at first become widely distributed, while the unraveling of the inner parts of it are naturally delayed, and the longitudinal separation of the two independent parts is observable in the portions of the spirem which are widely uncoiled (*fig. 20*).

The spirem becomes considerably thickened, certainly due to shortening, and becomes distributed loosely through the cavity; while the line of the longitudinal splitting or separation of the spirem into two elements seems obliterated, but its presence is evident in some places (*fig. 21*).

The course of the spirem again becomes irregular, and in some parts there are present complicated tangled masses of the spirem on account of the twisting of the spirem itself, but double threads, the constituents of the spirem, run mostly in close association (*fig. 22*). Shortening and thickening of the spirem proceeds until it segments into chromosomes. As shown in *figs. 23a* and *23b*, the segmentation is not simultaneous throughout the entire spirem, but finally there are formed 64 or 66 chromosomes, each bivalent in nature (*figs. 24a, b, c*).

The two halves of the bivalent chromosomes lose their close association after their segmentation, and show every possible position and connection with each other, such as H, I, J, K, L, O, T, U, V, X, Y, and so forth, and even give an impression of being of different length and size, mostly due to the point of view. The chromatin material which constitutes the surface region of the chromosomes seems loosely aggregated, but it is compactly massed in the center, and there are observed faintly stained substances of fibrillar form attached at the ends of chromosomes, which seem to be remnants of the material of the spirem left over at the time of segmentation, probably on account of the viscid nature of the spirem. The loosely aggregated chromatin material is gradually drawn and condensed toward the central part, but the condensation process takes place irregularly, so that for a while the chromosomes appear spiny. The chromosomes become shortened, and the loose fibrillar appendages are also shortened by contributing material to the main body of the chromosome, yet some can still be seen (*figs. 25a, b, c*).

A short description of the chromatic substance seems necessary. Early in the resting stage of the nucleus, the cytoplasm shows a fine fibrillar structure, almost homogeneously distributed. When the nuclear structure begins to enter into synapsis, long kinoplasmic fibers appear from the rearrangment of the cytoplasm surrounding the nucleus, but far apart from the nuclear membrane. Between these kinoplasmic fibers and the nuclear membrane there is present a clear region free from any fine fibrillar structure, which was the main feature of the other parts of the cytoplasm before the appearance of the kinoplasm (*fig. 21*). Some of the stages showing such a process are represented in *figs. 21–25*. The nuclear membrane, which has stained distinctly, now becomes less and less affected by stains and seems to be dissolved entirely, but for a short time the encroaching and anastomosing kinoplasmic fibers preserve the previous nuclear outline (*figs. 25a, b, c*). The kinoplasm, as a rule, surrounds the nuclear membrane, but some of the threads run in somewhat radial directions. No distinct polar formation was observed.

When the kinoplasmic fibers push into the nuclear cavity, some of them are seen to be attached to the chromosomes, while others pass through, and a multipolar structure becomes noticeable (*figs. 26a, b*); from the fibers of the multipolar spindle there is worked out the bipolar spindle, as indicated in *figs. 27a, b*. Shortening of the chromosomes continues always up to this time, so that now there result very short bivalent chromosomes, different in aspect from those in the early prophase. There is no regularity in the position of the chromosomes, which are found on almost all parts of the spindle, yet it is not hard to notice from successive stages, as *figs. 26a, b* and *27a, b*, that there is a tendency to move toward an equatorial region until 64 or 66 chromosomes are arranged in the equatorial plate.

The two parts of the bivalent chromosome in the equatorial plate are applied to each other throughout their entire length; ends in which spindle fibers are attached become directed toward the poles, while the other ends remain in contact. The polar view of the equatorial plate or a little earlier stage is favorable for counting the chromosomes, which showed always 64 or 66, each bivalent in nature (*fig. 29*).

SEPARATION OF THE DAUGHTER CHROMOSOMES.—Now the daughter

chromosomes begin to separate; some of them, on the way toward the pole, show a V-shape, the angle being directed toward the pole. This does not seem to have resulted from the bending of one daughter chromosome, because it always remains straight after separation; hence the V-shape may be regarded as resulting from a new longitudinal fission of the daughter chromosome, providing for the two granddaughter chromosomes of the second division. This longitudinal fission occurs only at the end of the chromosome directed toward the equatorial region, without proceeding to the other end. The two arms of the V come into contact again as the daughter chromosome reaches the pole.

As a rule, the sets of daughter chromosomes pass to the poles simultaneously, but there were observed some exceptional cases where some were much delayed (*figs. 31, 32*); and rarely the irregularity is so extreme that tardy chromosomes are left behind, while their associates are already in an aggregated condition at the pole (*fig. 34*).

Reconstruction of the daughter nuclei.—When the group of daughter chromosomes reaches the pole, each chromosome for a short time remains fairly straight, which is favorable for counting the chromosomes from the polar view (*fig. 35*). Then the vacuolization process begins (*fig. 36*). There is a certain point of difference in detail between this process and that described in the reconstruction of daughter nuclei in vegetative mitosis; the polar ends of the daughter chromosomes do not seem to be drawn together so as to give a concave form, but begin to be vacuolized at once, while in a state of loose association. The vacuolization is prominent along the line of the longitudinal fission of the daughter chromosomes, a line which became obliterated when the chromosomes reached the pole. After the formation of the nuclear membrane it is not impossible to recognize a certain part of the chromatin substance as representing a certain chromosome (*fig. 38*); in other words, the vacuolization process in the telophase of the heterotypic mitosis does not cause the chromosomes to return into the ragged chromatin reticulum from which they are formed, but the reconstruction stops before the individual outline of the main body of the chromosome is lost entirely. Therefore, the daughter nucleus does not pass into the resting stage with ragged

chromatin reticulum in the telophase of the first division, but always shows the main body of individual chromosomes recognized in V-shape, notwithstanding the nucleus grows considerably in size after the formation of the membrane. No nucleolus was observed in the daughter nucleus (*figs. 40, 41*). The nucleus in such a condition is ready for the second mitosis.

GRANULAR ZONE DIVIDING THE SPORE MOTHER CELL.—During the nuclear division described above, the cytoplasm generally has a fine fibrillar structure, with vacuoles here and there near the cell wall. When the chromosomes are arranged at the equatorial plate, fibers running from the poles cross in the equatorial region near the cell wall, where the cytoplasm begins to be transformed into the granules of this stage. After the organization of the daughter nucleus, the granules begin to increase and proceed rapidly toward the center along an equatorial plane of uniform thickness (*fig. 38*), at first avoiding only the space which was occupied by the central spindle (*fig. 39*). At last even the middle parts of the central spindle become replaced with or transformed into granules, so that finally the protoplast of the spore mother cell is entirely separated by a zone of these granules into two hemispheres, each with its daughter nucleus. The greater portion of the central spindle, which contributes to the formation of the granules, returns into the fibrillar structure of the cytoplasm at telophase.

CHROMOSOMES OF HOMOTYPIC DIVISION.—As described above, the daughter chromosomes which entered into the reconstruction of the daughter nuclei do not lose their individuality, but it is possible to recognize their identity even after they are combined and anastomosed together into a reticular structure (*fig. 41*). The recognizable V elements in the nucleus of the second division are what undoubtedly resulted from the influence of the vacuolization on the line of the longitudinal fission of the daughter chromosomes in the first mitosis during the last telophase; however, the arrangement of V's which they had when vacuolization began at the last telophase does not seem to have been maintained, for the closed angles of the V's are now visible on both upper and lower sides of the nucleus from such a side view as shown in *fig. 42*. Such a change in the situation of V's during the course of the vacuolization process and nuclear growth might easily

occur because of the bending and curving due to their being gathered into the round cavity of the daughter nucleus.

As the disappearance of the nuclear membrane approaches, V elements become more distinctly pronounced (*fig. 43*), some of the fine strands which branched out from V's disappear, but irregularity in thickness persists and the arms of the V's end in very slender threads. The general outline of the nucleus up to this time is always flattened, so that the nucleus shows two different aspects according to whether the view is from the pole or equator of the spore mother cell; hence the same mitotic figure of this nucleus, no matter what stage it may be, will always present two different side views, one being always narrower than the other.

As the nuclear membrane breaks down, the cytoplasmic fibers enter into the nuclear cavity, where the chromosomes are crowded (*fig. 44*). The figure of this stage recalls the combined features of both the heterotypic and the typical forms: the chromosomes, long, slender, and curved, with daughter chromosomes in pairs, resemble those of the vegetative mitosis; the achromatic fibers do not construct a cap, but appear multipolar as in the heterotypic case, although they are from the start observed near the nuclear membrane instead of at a distance from it. The two arms of the V's, which now provide for the two daughter chromosomes of this division, are still hanging together at the angle (*fig. 44*) at which spindle fibers seem to become attached. When the multipolar condition has passed into the bipolar spindle, the angle ends of the V's, or the ends where spindle fibers are attached, occupy the position near the pole, while the other ends diverge toward the equatorial region (*figs. 45, 46*). The stage resembles that represented in *fig. 3* as one of the transient conditions in passing to the stage of the equatorial plate.

Gradually the divergent arms of the V's, or the daughter pair of the bivalent chromosomes in this division, draw near to each other until finally they come into close contact. Simultaneously, the bivalent chromosomes, each consisting of two daughter chromosomes, move toward the equatorial region; as the ultimate result, the ends of chromosomes to which fibers are attached are arranged in an equatorial plane, while the other free ends are directed toward the pole (*fig. 47*). The figure of this stage is also comparable with that of the

vegetative mitosis shown in *fig. 4*. The shortening and thickening of the bivalent chromosomes begin and proceed in similar rate and degree in each member of the pairs, which is always in contact with its fellow (*figs. 48, 49*). These processes are exactly similar to those which have already been described in the vegetative mitosis. The bivalent chromosomes are finally shortened into straight, rod-shaped chromosomes and become arranged in an equatorial plate (*fig. 50*). Some of the bivalent chromosomes in this stage of the second division, unlike that of the first division, are slightly above or below the plane of the rest, so that the polar view is not satisfactory for accurate counting (*fig. 51*).

The separation of the daughter chromosomes which constitute the bivalent chromosomes begins at once. Generally daughter chromosomes pass in a group toward the pole, but occasionally the separation of the two halves of certain chromosomes is more delayed than the rest (*figs. 52, 53*). In any event, the daughter chromosomes which are on the way toward the pole have a straight rod shape, a little more elongated than they were when arranged in the equatorial plate. When the daughter chromosomes reach the pole they keep for a while a regular and almost parallel arrangement (*figs. 54, 55*), which was advantageous for counting. The number is 64 or 66 (*fig. 56*).

The group of chromosomes becomes more and more closely aggregated (*figs. 58, 59*). When the vacuolization begins, the individual outlines of the chromosomes are gradually lost, and after the organization of the nuclear membrane, which is formed as in the vegetative mitosis, the growth of the nucleus follows (*fig. 60*).

Simultaneous with the reconstruction of granddaughter nuclei the cell plate is laid down between them, exactly as in the vegetative mitosis (*figs. 57–60*). The second division forming two granddaughter nuclei takes place in the daughter nuclei of both hemispheres, separated already by the granular zone in the first mitosis. From the cytoplasm which filled the space between two granddaughter nuclei new spindle fibers now arise connecting them. These fibers necessarily pass the granular zone, in the middle plane of which is established the cell plate that really divides the two daughter protoplasts of the heterotypic mitosis (*fig. 61*). As a very rare case, the granular zone is not in a plane, and the fibers which connect the granddaughter nuclei of

different hemispheres arise before cell plates are laid down between them, so that the protoplast of the spore mother cell in this case becomes divided simultaneously into four tetrahedral spores.

Discussion of cytological phenomena

NUCLEAR RETICULUM.—Since the terms "chromatin" and "linin" were given by FLEMMING (**21**) and by SCHWARZ (**42**) respectively to the substance of the nuclear network which stains deeply and to the substance of the achromatic reticulum, these two substances have been regarded as essential constituents of the nucleus in its resting stage, and it became the general formula in describing the resting nucleus to state that it contains a fine network of linin fibers in which are imbedded chromatin granules.

To determine the real nature of chromatin and linin, and to find out the origin and differentiation of these two elements needs special investigation, which is not, of course, the object of the present work; however, the observation of events that occur between the telophase of one mitosis and the prophase of the next gave the writer the following notion regarding the nature of chromatin and linin, similar to the conclusion drawn by GRÉGOIRE and WYGAERTS (**25**).

When a group of daughter chromosomes reaches the pole, they are first drawn tightly together, so as to lose, apparently, their individual outlines. Vacuolization then takes place in this mass, and with the appearance of vacuoles parts of the mass of chromatin substance become irregularly separated and distributed so as to become transformed into a ragged reticular structure consisting of anastomosing, irregular clumps and finer, branched strands. The membrane, which may be formed by the contact of the surrounding cytoplasm with the nuclear sap that appears in the vacuoles, separates the mass of chromatin substance from the cytoplasm outside at a very early period, so that it is highly probable that no achromatic structures are included within the nucleus. The nuclear reticulum of the resting stage is nothing but the result of further advancement of the vacuolization. The new spirem of the ensuing division is formed again by the rearrangement of the ragged chromatin reticulum.

From the foregoing it seems quite probable that the nuclear reticulum is derived entirely from the chromosomes, the lightly staining

threadlike strands in the resting nucleus being only a part of the chromatin substance that has assumed such a structure.

REDUCTION OF CHROMOSOMES AND SYNAPSIS.—Since tetrads were first figured by HENKING (28) in the maturation division of an insect, several authors have reported the tetrad formation in various animals in connection with the reduction of chromosomes. From studies of the plant cell, the presence of the tetrads in the spore mother cell was independently described by OSTERHOUT (35) and CALKINS (11). OSTERHOUT figured the tetrads in the prophase of sporogenesis in Equisetum. CALKINS found in Pteris and Adiantum that the tetrads arise from rings or double rods, parallel or crossed, which resulted from the longitudinal splitting of primary chromatin rods or segments and which break up into four parts (the tetrads), followed by a transverse division. An essentially similar mode of tetrad formation was described by BELAJEFF (5) in Iris and by ATKINSON (4) in Arisaema and Trillium.

As already described, there is no tetrad formation in Nephrodium, chiefly due to the fact that both first and second divisions do not occur in rapid succession, and that the appearance of the fission providing for the second division is delayed until after the separation of the bivalent chromosomes of the first mitosis. At present most investigators believe that tetrads, in a strict sense, are not formed in the majority of cases, excepting in a few groups of animals.

The nature of these two successive divisions of chromosomes in the spore mother cell has been discussed by many authors, chief attention being paid to phases after the segmentation of the spirem into a certain number of chromosomes. Among early observers there has been every possible variation of opinion. Miss SARGANT (39) found in Lilium that both divisions were longitudinal. This view was soon concurred in by STRASBURGER (47), FARMER and MOORE (17), and others. Two years later STRASBURGER (50) and MOTTIER (33) concluded that the fission of the chromosomes in the second division was transverse, and essentially similar results were obtained by ISHIKAWA (30) in Allium, and by BELAJEFF (5) in Iris. DIXON (13,14,15) and SCHAFFNER (40, 41) held a different view, believing that the continuous thick spirem twists up into loops that are the incep-

tion of chromosomes, and that the chromosomes of the first division result from the loops, separating by a transverse division at the angle of the loops; while the second division is longitudinal. ATKINSON (4) found two different methods in Arisaema and in Trillium; in the former species the first division was transverse, as DIXON and SCHAFFNER found; while in the latter the second was transverse, as STRASBURGER and MOTTIER claimed.

Among these three divergent views, which claim respectively that there occur two longitudinal divisions, that the first division is transverse and the second longitudinal, and that the first division is longitudinal but the second is transverse, the third was of short duration. The authors (STRASBURGER and MOTTIER) who described the existence of such a case changed their opinion and returned to their former view that both divisions are longitudinal, so that there persist only two views, one claiming the presence of two longitudinal divisions, and the other claiming that the first is transverse: the former one of these was held by GUIGNARD (27), GRÉGOIRE (22), LLOYD (31), and others.

However, the more recent studies of synapsis have involved very detailed and accurate observations concerning the nature of the reduction division. Some of the investigators mentioned above have changed their views, while others still maintain them, but in both cases a new or modified notion concerning the origin of chromosomes and the nature of reduction division is held.

As described before, the nucleus of Nephrodium in the presynaptic stage consists of a complex, anastomosing ragged chromatin reticulum. This ragged reticulum shows a tendency to become transformed into a thread structure, but the process does not occur simultaneously in different regions. When the transformation has taken place, the two parts of the thread are observed running side by side from the first. Such a condition as is represented in *figs. 16* and *16a* is evidently what was called "leptonema" by WINIWARTER (51). Further transformation of the thread structure from the ragged reticulum results in a nucleus with a continuous chromatin thread or spirem whose double nature is only visible at certain parts on account of the close association. The thread becomes tangled and contracted in one side of the nuclear cavity (*figs. 17* and *17a*) until finally there results

the climax of the synaptic stage (*figs. 18, 19*); these two stages may cover the "zygonema" condition of WINIWARTER.

The above brings the writer to the conclusion that the spirem is of double nature from the outset, probably derived from the paternal and maternal source, as was interpreted by ALLEN (**1, 2, 3**), GRÉGOIRE (**25**), BERGH (**6, 7, 8, 9**), ROSENBERG (**37, 38**), and CARDIFF (**12**).

In preparations stained with iron-alum-hematoxylin and extracted so as to obtain a proper differentiation, the paired chromatin threads of the spirem appear as if consisting of darkly stained granular parts and a lightly colored ground-substance of threadlike structure, but this simply means that they are not of homogeneous thickness; the more aggregated the chromatin substance, the more dark appears the stain, and therefore probably the individuality of the two halves of the spirem may not be lost entirely during the close association.

There are observed in Nephrodium a number of parts of the spirem running through the contracted mass from the nuclear membrane where the mass lies in contact (*fig. 18*). GRÉGOIRE (**24**) found that a similar condition of the spirem exists in Osmunda, and that in a later stage the chromosomes resulting from the thick spirem held a similar orientation in regard to the nuclear membrane. From this he concluded that there is no continuous thick spirem in the prophase of heterotypic division of Osmunda. Since in Nephrodium the thick spirem as it emerges from synapsis is always in contact with the nuclear membrane up to the segmentation of chromosomes, the condition shown in *fig. 18*, where the number of parts of the spirem run out from one side of the membrane, may have some significance which we are unable to interpret at present.

The thick spirem emerging from synapsis showed first, at the regions widely uncoiled from the contracted mass, longitudinal fissions which came undoubtedly from the separation of the two independent threads that entered into association during synapsis. The thick spirem simply segments into 64 or 66 bivalent chromosomes, each of the pairs being derived from the independent thread. In a certain stage before the "strepsinema" condition, appearances of looping were also observed in connection with the contracted region, but they had nothing to do with the formation of chromosomes as

described by FARMER and MOORE (**17, 18, 19**), GREGORY (**26**), FARMER and SHOVE (**20**), MOTTIER (**34**), and SCHAFFNER (**41**).

Consequently, the reduced number of chromosome segments of the heterotypic mitosis in Nephrodium are not two sporophytic chromosomes joined end to end, which come to lie side by side by the approximation of the arms of the loops and break apart at the head of the structure; but they are bivalent chromosomes or pairs of sporophytic chromosomes derived from the two threads of the synaptic stage that are always in close association side by side from the beginning, as claimed by ALLEN, BERGHS, and GRÉGOIRE.

Results obtained by MIYAKE (**32**) in a number of monocotyledons and by OVERTON (**36**) in a number of dicotyledons accord in general with this description of the mode of formation and separation of heterotypic chromosomes.

Although the present account of Nephrodium differs widely from the accounts of FARMER and MOORE, GREGORY, STEVENS, and STRASBURGER in the interpretation of the synapsis and the prophase of heterotypic mitosis, yet it agrees in one fundamental point, namely, that the first division is heterotypic and separates whole sporophytic chromosomes.

HOMOTYPIC DIVISION.—That the chromosomes in the second division in Nephrodium divide longitudinally is in accordance with the vegetative mitosis; however, there exists an essential point of difference between the two cases, namely, in vegetative mitosis the resting stage is always intercalated between the chromosomes in the last telophase and those of prophase of the ensuing division, so that each mitosis is followed by a resting stage, and a line of new longitudinal fission of the chromosomes arises in the early prophase of each division; while in the homotypic mitosis no resting nucleus is formed in telophase of the preceding mitosis, and therefore the homotypic mitosis should not be regarded as a complete one, standing by itself. Really an early part of the prophase of the second division may be regarded as being initiated at the first division, because the homotypic mitosis separates two daughter halves of a single sporophytic chromosome of the first division; the partial longitudinal fission of this sporophytic chromosome was begun during the anaphase of the first mitosis, although the fission was obliterated in the last telophase.

According to the count of chromosomes which was made in the telophase of the first division, the daughter nucleus may contain 64 or 66 chromosomes, the reduced number; however, it should be remembered that this is certainly due to the obliteration of the longitudinal fission which appeared in the anaphase, and since this early part of the event of the second mitosis is actually initiated in the anaphase of the first division, the daughter chromosomes should well be considered as bivalent, consisting of two granddaughter chromosomes, although the actual separation is completed in the prophase of the second division. Therefore the homotypic division, in spite of its dependence upon the first division, should be regarded rather as a real part of the reduction division, which completes the process initiated in the heterotypic mitosis; in other words, the reduction division is initiated in the heterotypic mitosis, but the reduction of the first division is only apparent; the real reduction in the number of chromosomes is completed in the telophase of the second division. It follows that the first nucleus which contains the reduced number of chromosomes is the spore, and consequently the spore marks the starting-point of the new generation, as discussed by GRÉGOIRE (**25**).

CENTROSOME.—The presence of the centrosome among the pteridophytes was reported in the spore mother cell by HUMPHREY (**29**) in Psilotum and Osmunda, and by CALKINS (**11**) in Adiantum and Pteris. OSTERHOUT (**35**) denied the presence of the centrosome in the spindle formation of Equisetum.

In Nephrodium, the spindle always arises from the rearrangement of cytoplasm. In sporogenesis a multipolar polyarch spindle is first observed when the nuclear membrane disappears, while in typical mitosis in the vegetative cell, two kinoplasmic caps first appear at the poles of the nucleus before the disappearance of the nuclear membrane. In both cases when chromosomes are arranged in the equatorial plate, spindle fibers gather into a focal center which lacks the centrosome or centrosphere, and the gathering of the fibers into the focal center persists until the last anaphase or early telophase; however, in no stage of mitosis are there present centrosomes, radiations, centrospheres, or any kind of structure which was believed to control nuclear division. As regards the mechanism of mitosis, the writer is not prepared to enter into any discussion. The centrosome question

in connection with the blepharoplast in pteridophytes will be discussed in the next paper, which will deal with gametogenesis and fertilization in Nephrodium.

UNIFORMITY IN THE NUMBER OF CHROMOSOMES.—STRASBURGER'S paper (**46**) entitled "The periodic reduction of the number of the chromosomes in the life-history of living organisms," which was the first enunciation of the significance of sporogenesis and the reduction phenomena in relation to the alternation of generations in plants, was based upon the discovery that there is a uniformity in the number of chromosomes in certain phases of the life-history of the individual. Evidence seems to be accumulating from different groups of plants confirming the notion of constancy in the number of chromosomes in individuals under normal development, although there are instances where different authors report different counting.

Generally speaking, accuracy in counting the chromosomes depends upon the number and form of the chromosomes in the individual under consideration. When the number of chromosomes is large and their form is slender and curved, accurate counting is naturally difficult. In Nephrodium the writer found this difficulty, due to the great number and curved form of the chromosomes, but it was not impossible to make reliable counting by using certain stages in which the chromosomes assumed a straight rod shape and a regular arrangement, characteristic of this species. In sporogenesis there are three conditions favorable for counting. First the prophase after the segmentation of the spirem into chromosomes, up to the stage just before the disappearance of the nuclear membrane, was favorable for counting It might well be expected that two parts of the same chromosome which happened to be cut might appear in two successive sections of the same nucleus, and this would make the counted number larger than the actual. Nevertheless, after carefully examining the mitosis in this stage, it was possible to find out the sections with the least number of cut chromosomes, sometimes only two, as was given in *figs. 24a, b, c* and *25a, b, c, d.* The second condition good for accurate counting is the polar view in the early metaphase; and the third case is the polar view at a certain stage of the late anaphase, in which the chromosomes are arranged near the pole.

In the typical mitosis of the vegetative cell, on the other hand, the

prophase previous to the disappearance of the membrane is not good for counting, because of the curved and twisted nature of the chromosomes. The polar view of the late prophase or early metaphase is favorable for gross estimation, but in a certain critical stage of late anaphase, when the chromosomes are in irregular arrangement at the pole, accurate counting is always possible.

From the counting made in the stages mentioned above, the writer believes that there is a constant number of chromosomes in Nephrodium, i. e., 128 or 132 in typical mitosis and 64 or 66 in the reduction division; the result is evidently not in harmony with the conclusion drawn by FARMER and DIGBY (**16**) in their recent paper.

The reason for the presence of two sets of numbers of chromosomes (such as 64 or 66) has not been determined.

Conclusion

Since this study of sporogenesis was carried through as preparatory for the study of apogamy in Nephrodium, the general conclusions will appear in the final paper. Two points, however, may be summarized here: (1) there is uniformity in the number of chromosomes in the sporophyte; and (2) in the normal life-history there is a reduction of chromosomes in sporogenesis.

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EXPLANATION OF PLATES I–IV

All figures were drawn with the aid of an Abbé camera lucida, using Zeiss's apochromatic objective 1.5^{mm}, N. A. 1.30, combined with compensating ocular 18, except *figs. 15a, 16a, 17a,* and *19,* which were drawn with same combination of objective and ocular under higher magnification obtained when the tube was extended to the farthest point.

The plates are reduced one-half the original size, except *plate 1,* which is reduced two-fifths.

PLATE I

Vegetative mitosis in the sporogenous cell in the eight-cell stage

FIG. 1. The prophase; the spirem before segmentation into chromosomes; a kinoplasmic cap with a few fibers at each pole; the membrane beginning to disappear at those points.

FIG. 2. Spirem segmented into chromosomes; chromosomes long and curved, split longitudinally, the two halves running closely in contact.

FIG. 3. Chromosomes arranged parallel along the axis passing from pole to pole, one end of chromosomes in which spindle fibers are attached lying in an equatorial plate.

FIG. 4. The intermediate stage in passing from the previous condition to the stage of an equatorial plate; the chromosomes much shortened, one end where spindle fibers are attached being bent, while the end directed to the pole is straight.

FIG. 5. Metaphase, showing an equatorial plate; chromosomes short and rod-shaped.

FIG. 6. Late metaphase; the daughter chromosomes beginning to separate.

FIG. 7. Anaphase; two sets of the daughter chromosomes on the way toward pole; chromosomes almost straight, rod-shaped, slightly more elongated than metaphase.

FIG. 8. Late anaphase; rod-shaped chromosomes parallel at the pole.

FIG. 9. Polar view of stage given in previous figure, showing 128 chromosomes.

FIG. 10. Group of daughter chromosomes vacuolized, the upper side, for merly the pole, being concave.

FIG. 11. Polar view of stage shown in previous figure; arrangment of chromatin material resulting from vacuolization of chromosomes presents regularity.

FIG. 12. Daughter nuclei after formation of membrane, still of flattened form; cell plate has appeared in middle region of central spindles.

FIG. 13. Slightly oblique side view of daughter nucleus after formation of daughter cell; size increased; two nucleoli have appeared in the previously polar region.

FIG. 14. Polar view of daughter nucleus in later stage than that shown in previous figure; nucleoli apart from chromatin material.

PLATE II

Sporogenesis

FIG. 15. One of three sections of a resting nucleus in spore mother cell; chromatin reticulum without any regularity, looking ragged; three nucleoli are present in this section, some of them vacuolate.

FIG. 15*a*. Portion of chromatin reticulum of previous figure under higher magnification; reticulum consists of irregular clumps and slender strands, giving ragged aspect.

FIG. 16. One of three sections of a nucleus in which is shown the tendency to form the thread structure from a rearrangement of the chromatin reticulum; a single nucleus is seen in this section.

FIG. 16*a*. Portion of chromatin reticulum from *fig. 16* under higher magnification; by the disappearance of some of the strands and elongation of ragged clumps, thread structure is formed; these threads from the outset independent.

FIG. 17. Early synaptic stage; chromatin reticulum, almost transformed into continuous thread, begins to contract at one side of the nuclear cavity.

FIG. 17*a*. Portion of the parallel threads in *fig. 17* under higher magnification.

FIG. 18. Synapsis; nucleolus caught in the contracted mass of thread at a position not in contact with the nuclear membrane.

FIG. 19. Portion of the separating threads from a tangential section, the next to that shown in *fig. 18*, under higher magnification.

FIG. 20. The nucleus emerging from synapsis; uncoiled parts of the spirem show the longitudinal splitting or separation of two threads which entered into close association in synapsis.

FIG. 21. Spirem shortened, thickened, and more uncoiled; line of longitudinal splitting or separation visible; kinoplasmic fibers present surrounding the nucleus, circumscribing the comparatively clear zone near the membrane.

FIG. 22. Spirem more thickened and distribution more irregular than in the preceding stage shown in *fig. 21;* kinoplasmic fibers approaching nearer to the membrane.

FIGS. 23*a*, 23*b*. Two sections of the same nucleus when chromosomes begin to be segmented; two halves of the spirem almost equally thickened and in close association; spirem irregularly contracted.

FIGS. 24*a*, 24*b*, 24*c*. Three sections of the same nucleus after segmentation of spirem into 66 bivalent chromosomes; association of two halves of each chromosome has become somewhat loose, so that various combinations of the two daughter halves are shown, such as H, I, J, K, L, O, U, V, X, Y, and so forth; thin strands of chromatin material visible attached to the ends of chromosomes; two chromosomes (*a* and *b*) are cut into two, so that the two parts of the each chromosome appear in two successive sections.

FIGS. 25*a*, 25*b*, 25*c*. Four sections of the same nucleus; chromosomes show spiny structure; nuclear membrane has disappeared, the outline of nucleus being maintained by the encroaching and anastomosing of fibers; two chromosomes (*a* and *b*) are cut into two, so that the two parts of each chromosome appear in two successive sections.

PLATE III

Sporogenesis continued

FIGS. 26*a*, 26*b*. Two sections of the same nucleus, after intrusion of fibers; multipolar spindle is established.

FIGS. 27*a*, 27*b*. Two sections of the same nucleus, after the multipolar spindle has passed into the bipolar condition; bivalent chromosomes, two daughter halves always in pairs, have shortened considerably.

FIG. 28. Early metaphase, the bivalent chromosomes being regularly arranged in an equatorial plate.

FIG. 29. Polar view of early metaphase; lightly shaded chromosomes represent those which could be seen at a deeper focus; 64 bivalent chromosomes are present.

FIG. 30. Metaphase; daughter chromosomes begin to separate.

FIG. 31. A later stage than that shown in *fig. 30;* some of the daughter chromosomes which lie on the way to the pole indicate a new fission that provides for the second mitosis.

FIG. 32. Anaphase; rate of movement of daughter chromosomes toward the pole is variable.

FIG. 33. Late anaphase; some of the daughter chromosomes at the pole still show V-shape, due to the fission first indicated in *fig. 31*.

FIG. 34. Early telophase; daughter chromosomes are grouped at the pole, two of them being tardy; no V-shaped chromosome present.

FIG. 35. Polar view of stage shown in *fig. 34*, showing 64 chromosomes.

FIG. 36. Telophase viewed from the pole; chromosomes vacuolized; nuclear membrane constructed.

FIG. 37. Polar view of daughter nucleus in a later stage of telophase; vacuolization farther advanced.

FIG. 38. Daughter nucleus begins to grow in size; masses of the chromatin reticulum may represent main bodies of vacuolized chromosomes; granular structure begins to arise along periphery of equatorial region where fibers from poles meet.

PLATE IV

Sporogenesis continued

FIG. 39. Polar view of stage shown in *fig. 38;* granular zone surrounding cross-sections of central spindles.

FIG. 40. Spore mother cell separated into two hemispheres by granular zone; daughter nucleus has grown in size; V elements distributed in chromatin reticulum represent main bodies of vacuolized chromosomes.

FIG. 41. Polar view of daughter nucleus shown in *fig. 40.*

FIG. 42. Side view of nucleus of second division; a little later stage than that given in *fig. 41.*

FIG. 43. Nucleus in same stage as previous figure; only parts of chromatin reticulum visible in one focus are drawn; V elements distinct.

FIG. 44. Prophase of nucleus of second division after disappearance of nuclear membrane; spindle multipolar.

FIGS. 45, 46. Prophase after bipolar spindle is established; these two figures are in the same stage, the former being the view from the pole of the spore mother cell, the latter the view from the equator of the same.

FIG. 47. Divergent arms of V's, i. e., daughter halves of chromosomes of second division have become parallel, the ends where spindle fibers are attached lying in the equatorial region.

FIGS. 48, 49. Late prophase; the two figures represent the same stage, viewed from the equator and pole of the spore mother cell respectively.

FIG. 50. Metaphase; two daughter halves in each bivalent chromosome exactly similar in size and shape.

FIG. 51. Polar view of stage intermediate between the two conditions shown in *figs. 48* and *50,* this aspect being due to the overlapping of the chromosomes.

FIGS. 52, 53. Anaphase; two figures represent the same conditions viewed from the equator and pole of the spore mother cell respectively; two or three pairs of daughter chromosomes are tardy in separation.

FIGS. 54, 55. Late anaphase; the two figures show the same stage viewed from the equator and pole of the spore mother cell respectively.

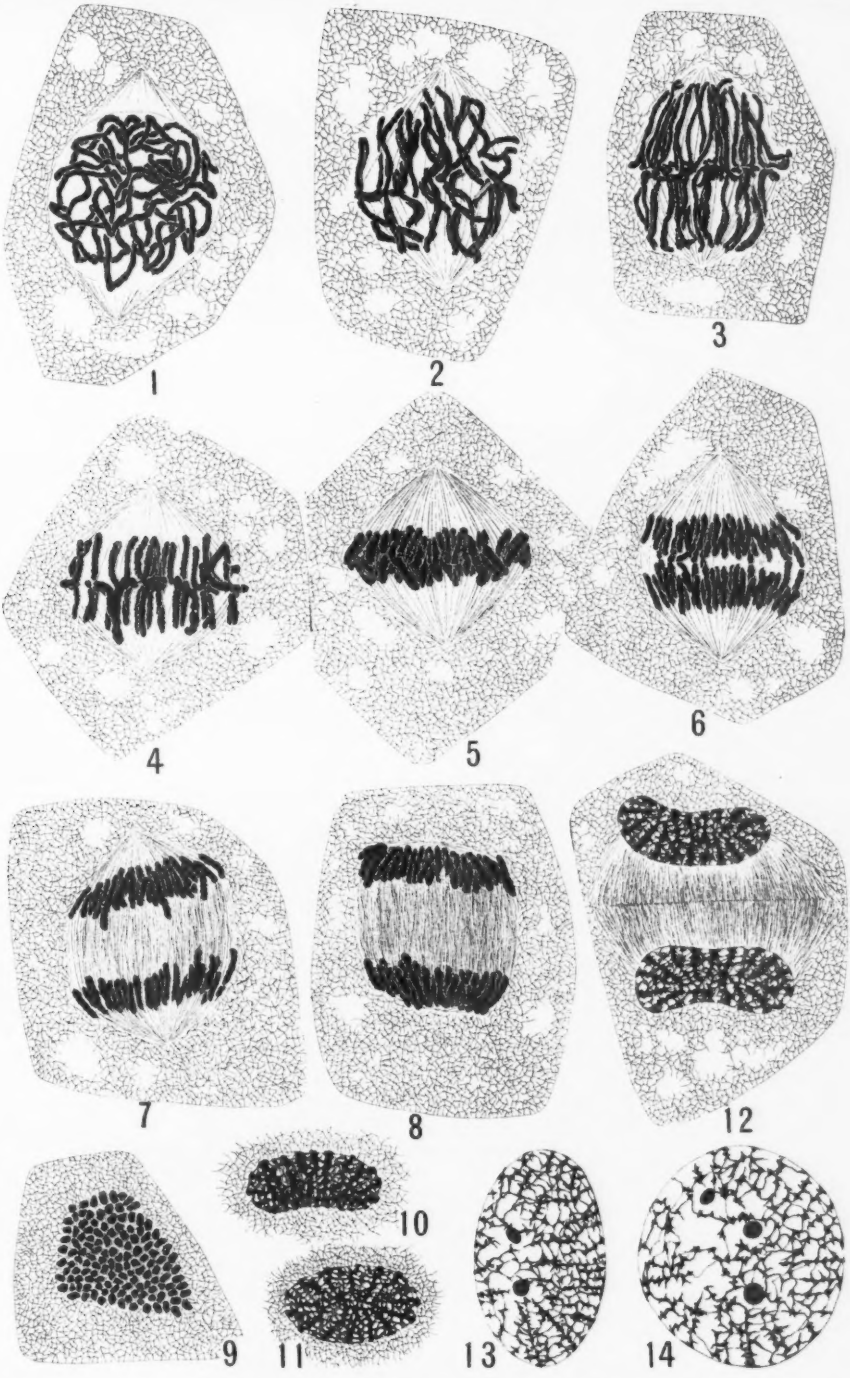
FIG. 56. Polar view of daughter chromosomes of late anaphase shown in *figs. 54* and *55;* 64 chromosomes.

FIG. 57. Early telophase; daughter chromosomes of second division at the pole and arranged regularly; cell plate laid down at middle region of central spindles.

FIGS. 58, 59. Telophase; the two figures show the same stage, viewed from equator and pole of spore mother cell; cell plate has divided the protoplast.

FIG. 60. Late telophase; membrane of the granddaughter nucleus formed; new fibers present between granddaughter nuclei of different hemispheres which were separated by granular zone in telophase of first division.

FIG. 61. Still later condition than stage shown in previous figure; cell plate completed through middle region of granular zone, thus dividing the two protoplasts separated in the first mitosis.



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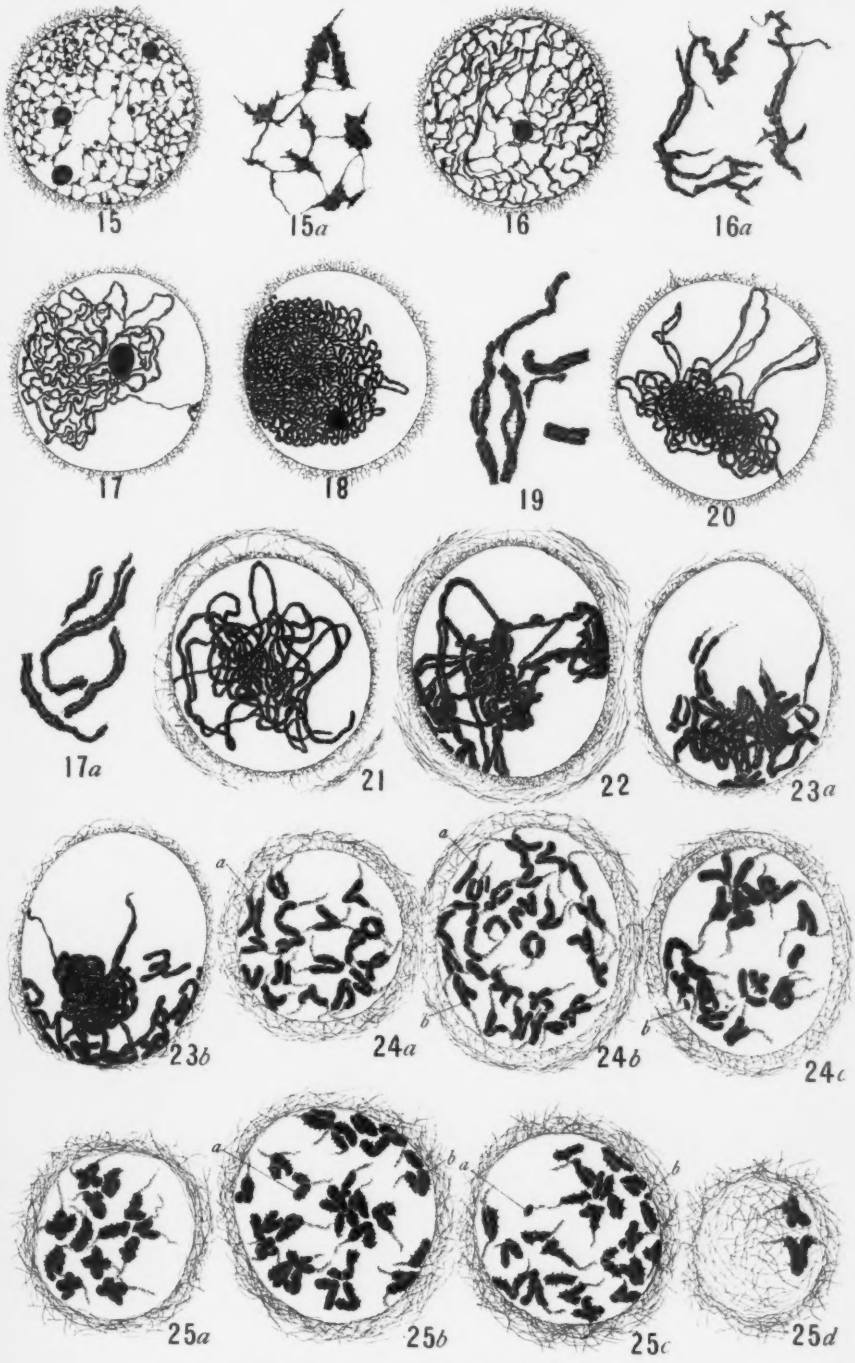

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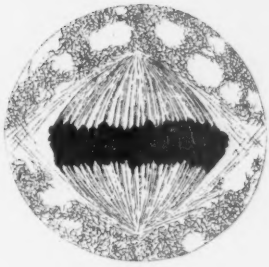
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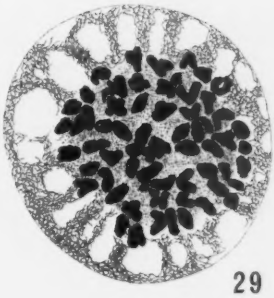
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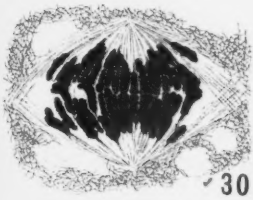
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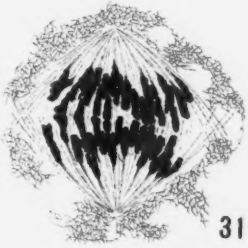
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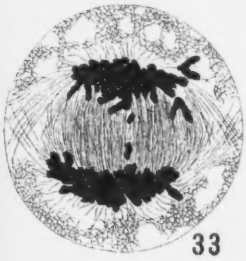
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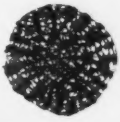
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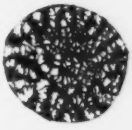
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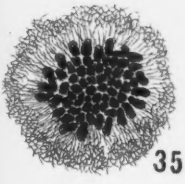
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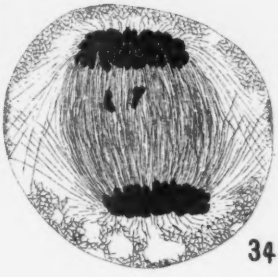
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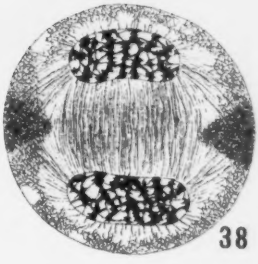
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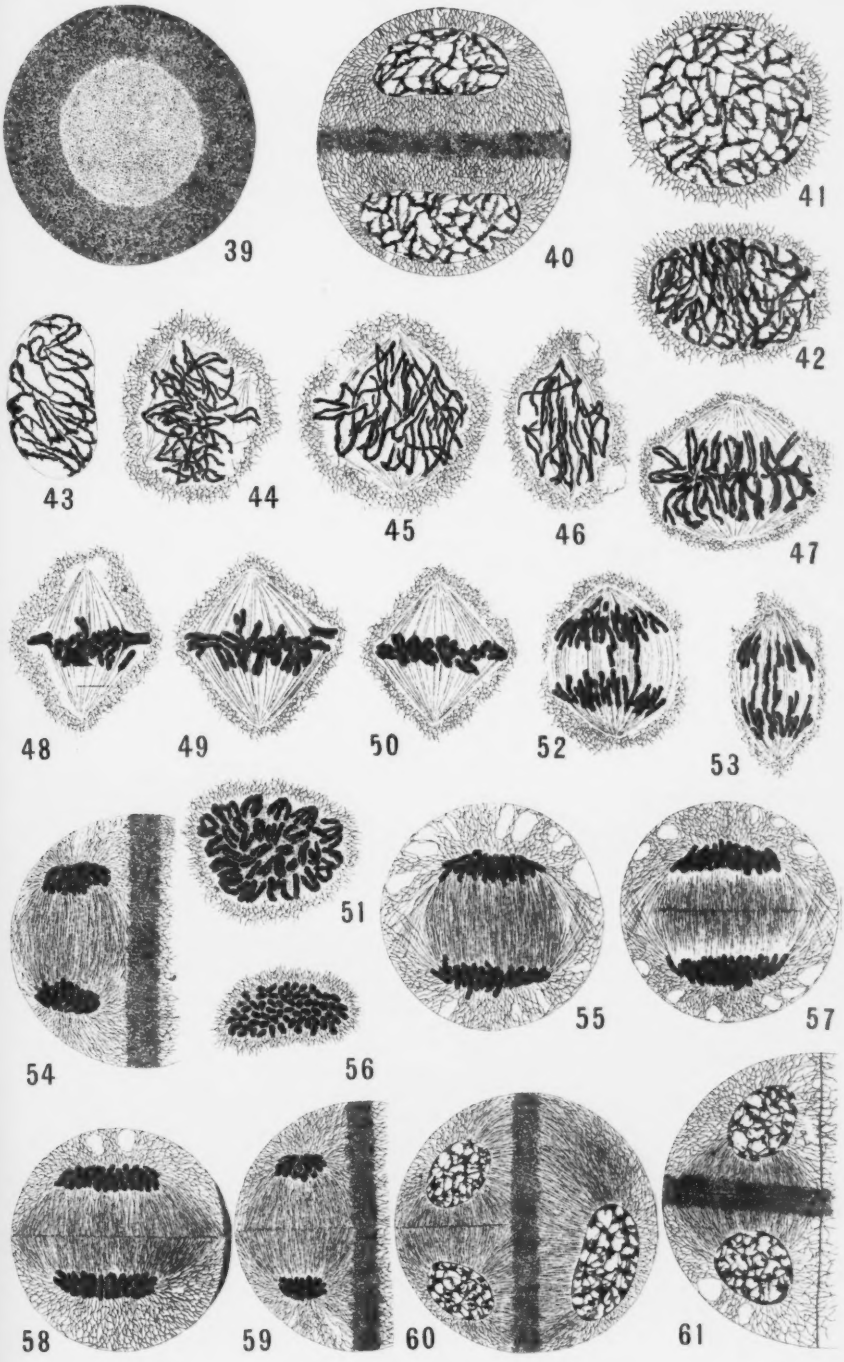
YAMANOUCHI on NEPHRODIUM



YAMANOUCHI on NEPHRODIUM

THE DEVELOPMENT OF THE POLLEN OF SARRACENIA

M. LOUISE NICHOLS

(WITH PLATE V)

During March 1907, through the courtesy of Dr. J. M. MACFARLANE, I had the opportunity of gathering material for this study from the fine collection of sarracenias in the greenhouses of the University of Pennsylvania. The species examined were *S. flava*, *S. purpurea*, *S. variolaris*, *S. rubra*, and *S. psittacina*, besides the hybrids *S. flava* × *variolaris* and *S. flava* × *purpurea*. A close similarity prevails in the pollen of the different species, nor does any marked difference appear in the hybrids. Abnormalities of division among the latter are practically absent.

The material consisted of flower buds fixed in chrom-acetic acid. Thin sections of the anthers were stained by the iron-hematoxylin method and with safranin and malachite green.

The nucleus of the pollen mother cells contains an intensely staining nucleolus surrounded by a clear space. With safranin and malachite green the color of the nucleolus is red. The surrounding plasm (linin) does not stain deeply, and shows a trace of fibrillar structure (*fig. 1*). The approach of the synapsis stage is indicated by a marked and apparently rather sudden growth in size of the nucleus. The nucleolus also becomes larger, and the clear space surrounding it less well defined but wider. The linin shows more definite indications of a network (*fig. 2*). A few deeply staining granules now appear within the linin; these increase in number as the network develops (*fig. 3*). Occasionally granules can be seen in close proximity to the nucleolus (*fig. 3*), which become larger and irregular in shape, and show a tendency to accumulate in the vicinity of the nucleolus and to surround it (*figs. 4–7*). In such nuclei there is sometimes evident a similarity in size of pairs of chromatin clumps. Thus in *fig. 4* there are visible two masses of chromatin, one on each side of the nucleolus of about the same size; while farther removed from the nucleolus there are two others, smaller than the first, but approximating each other in size. Similar relations are seen in *fig. 5*.

The chromatin next becomes densely massed around the nucleolus, and the irregular clumps elongate more or less into rods. So compactly do they come to lie around the nucleolus that it is completely hidden from view, and only at the margin of the somewhat rounded mass can a threadlike appearance of the chromatin be observed. The chromatin is now gathered together at one side of the nuclear space and the synapsis is well established (*figs. 7, 8*). The nucleolus gradually emerges from the center and takes a position to one side of the chromatin. When it has completely emerged its staining capacity is seen to have diminished (*figs. 9–12*). In some cases a bipartite arrangement of the chromatin is observable (*figs. 11, 12*).

The synaptic mass in the cells at one end of the anther sac now begins to lose its power to take the stain uniformly, and an appearance of dark clumps lying in a paler matrix is produced. The nucleolus is also usually vacuolated (*figs. 13, 15, 16*). The dark bodies approach the nucleolus and it seems to sink into or to be surrounded by the paler area. Within its substance is frequently seen material staining deeply like the bodies without; often this forms a partial ring around a vacuole (*fig. 16*). The nucleolus now becomes well filled with darkly staining material, while the substance without, which takes a similar stain, decreases in amount; it finally disappears entirely. At the same time the nucleolus has become entirely black with safranin red, and lies wholly within the paler substance (*figs. 17–19*). This condition was observed in *S. purpurea* and in *S. flava*×*purpurea*. The other species did not present suitable material for this stage.

The linin next begins to extend toward the circumference of the nucleus and to be converted gradually into a network (*figs. 20–22*). Dark granules, purple with safranin and malachite green, now appear in the network, and at the same time the nucleolus again shows vacuoles (*figs. 23, 24*). It seems to go through in a reverse order its former series of changes, dark material within the nulceolus often surrounding a central vacuole (*fig. 23*). Sometimes the nucleolus presents the appearance shown in *figs. 23a* and *23b*. At one focus three darkly staining spheres can be seen, whereas at a slightly higher level these spheres are replaced by vacuoles. In one case a body closely resembling the nucleolus was observed lying just outside the

nucleus, as if it had been accidentally displaced in cutting the section (*fig. 28*). If this be true, it would seem to indicate that the nucleolus is more or less independent in structure and has a resistant wall of its own. Occasionally, especially in *S. purpurea*, two nucleoli are found, one slightly smaller than the other (*fig. 24*). Sometimes (observed in *S. flava* and *S. variolaris*) the central portion of the nucleolus appears dark, the peripheral portion lighter, while from the central mass are budded off minute spheres (*figs. 22, 25*); or half the nucleolus may stain deeply, the other half faintly (*fig. 27*). The chromatic network eventually consists of fine granules distributed on delicate linin fibrils (*fig. 27*). At this stage the nucleolus, in preparations stained with safranin and malachite green, colors purple, inclosing darker purple granules, the vacuoles pinkish. The nucleoli of the tapetal cells, on the other hand, stain red.

The conversion of the network into chromosomes during the prophases of the first maturation division is illustrated in *figs. 29–31*. A longitudinal splitting of the chromatin granules and linin fibers is clearly seen, the halves thus formed often showing a tendency to spread apart (*fig. 30*). The chromosomes occur in pairs, which come to lie parallel to each other (*figs. 29–31*). They gradually shorten and thicken, so that finally little difference is evident between the length and breadth. That the spindle is formed, at least in part, from the linin fibers would appear from *fig. 32*, which represents a very late prophase. The spindle fibers when fully formed are sharply defined and show minute swellings. Two fibers may be seen attached to a chromosome in *fig. 33*. The separation of the daughter chromosomes is represented at its inception in *fig. 34*. The U-shaped chromosome would seem to correspond to the more elongated body of similar shape in *fig. 29*, and if this is true the first division separates univalent chromosomes and is reducing. The nucleolus persists at its full size until a late prophase. The total number of chromosomes in *S. variolaris* and in *S. rubra* is apparently twelve. The material did not present stages suitable for determining the number in the other species studied. SHREVE (**10**) reports twelve in *S. purpurea*.

After the first division of the chromatin, the daughter nuclei undergo no reconstruction, but arrange themselves for the succeeding mitosis (*figs. 36, 37*). The chromosomes seem to become somewhat

elongated and still manifest a longitudinal split. The second division separates the chromosomes along the longitudinal split and is therefore equational (*fig. 37*). The daughter nuclei are now reconstructed. A large dark nucleolus appears in each, while the rest of the nucleus is occupied by a linin network and scattered chromatin granules. *Fig. 38a* would seem to indicate that these granules are formed by the breaking-up of larger clumps. The nucleolus stains red, the network purplish with safranin and malachite green. During mitosis the chromosomes are bright red, but after reconstruction this color is gradually lost. The cytoplasm, which has remained undivided, now constricts and the four cells of the tetrad are formed (*fig. 40*).

DISCUSSION

THE NUCLEOLUS.—This term, in its limited sense, has been applied to bodies within the nucleus more or less spherical in shape and staining unlike the chromatin (WILSON **12**). The great diversity in the observations on the behavior of this body has given rise to several theories concerning its origin and function. MONTGOMERY (**6**), in his comprehensive review of the literature on this subject in both plant and animal cells, and WAGER (**11**), in his discussion of the nucleolus in plant cells, mention a large number of authors whose observations lead them to believe that there is a connection between the nucleolus and the formation of chromatin. This view is supported, not only by the morphological relations of nucleolus and chromatin, but also by researches on their chemical relations. Another opinion is that the nucleolus has something to do with spindle formation. STRASBURGER (**9**) and NĚMEC (**7**) adopt this view. Many writers on the Protista also report kinoplasmic material arising from the nucleolus. A third theory maintains that the nucleolus contains material which is periodically expelled into the cytoplasm. By some, notably HÄCKER (**3**), this is considered an excretory function. Others believe that the phenomenon is correlated with the appearance in the cytoplasm of substances having there a definite physiological duty.

My own observations afford support to the first of the explanations outlined above. The growth in size of the nucleolus simultaneously with the appearance of darkly staining granules in the nucleus, the accumulation of this material in the nucleolus, and its expulsion,

lead irresistibly to the conclusion that the formation of the chromatin is intimately connected with nucleolar activity. The relations of the nucleolus and the chromatin in the prophases of the first maturation division again plainly suggest an elaboration of chromatin by the nucleolus. The fact that at this time the nucleolus stains purple, whereas ordinarily it stains red with the safranin and malachite-green combination, indicates that a chemical change of some sort is taking place within it. The globules of material thus elaborated escape into the nuclear sap, there to be absorbed by the linin and distributed along its threads. GREGORY (**2**) was led to a somewhat similar view by his study of the pollen development in sweet peas. His observations indicate that "the nucleolus receives and stores the bulk of the chromatin during the resting periods which intervene between successive mitoses."

The varying observations concerning the behavior of the nucleolus give rise to the opinion that it has not the same definiteness of function as the chromosomes and centrosomes. Since there is certainly an exchange of material between cytoplasm and nucleus, and within the nucleus progressive and regressive changes in chemical constitution of its contents, it would seem that a rounded body staining unlike chromatin and therefore designated nucleolus might frequently arise, but representing at different times and in different cells a different physiological activity. The precise nature of the metabolism of the cell, it is natural to suppose, varies according to the kind of cell and the species of plant or animal, and therefore the nucleoli may be expected to vary in appearance and in their relation to the chromosomes or other constituents of the cell. That this may be the case in the ova of related species of animals has been demonstrated by MCGILL (**4**) in her studies of the dragon flies. In some forms apparently the nucleolus elaborates material for the use of the chromatin and thus granules and irregular masses occur within it. In others perhaps the material is not greatly transformed in the nucleolus, but passes out into the nuclear sap there to be absorbed and utilized by the chromatin. In the latter instance the connection between the nucleolus and the formation of chromatin would be difficult of demonstration. Thus such evidence as is given by DUBLIN (**1**) of the non-participation of the nucleolus in chromatin formation is negative

in character, and does not necessarily disprove this function of the nucleolus.

SYNAPSIS AND THE INDIVIDUALITY OF THE CHROMOSOMES.—The chromatin in this stage is so densely massed together as to make it impossible to distinguish individual chromosomes and to determine whether or not there takes place a conjugation of chromosomes as required by MONTGOMERY's hypothesis. According to the researches of MIYAKE (**5**) and OVERTON (**8**), supporting STRASBURGER's observations, the chromosomes are preceded in the pollen mother cells by prochromosomes, centers around which the chromatin collects, and which correspond to the chromosomes in number. In Sarracenia I was unable to satisfy myself that the points around which the chromatin collects were constant in number or that the number was the same as that of the chromosomes.

The behavior of the nucleolus just described is not entirely favorable to the idea of the individuality of the chromosomes as such. If any morphological differentiation of the nucleus into areas corresponding to the chromosomes really exists at this time, its basis might possibly lie in the linin. The chromatin and nucleolus at the stage represented in *fig. 19* stain red with safranin, while the linin takes the green. This stain serves to bring out a fibrillar structure in the linin. It is well known that the chromosomes differ greatly in staining capacity at different periods in their development. At certain stages they seem to lose a part of their substance and to diminish in size as well as in staining capacity; whereas at another period they are able to absorb material, grow in size, and color more deeply. The phenomena described might therefore be explained on the assumption that the morphological basis of the chromosomes remains in the linin, while that part of their substance which causes them to color deeply is absorbed by the nucleolus.

PHILADELPHIA NORMAL SCHOOL

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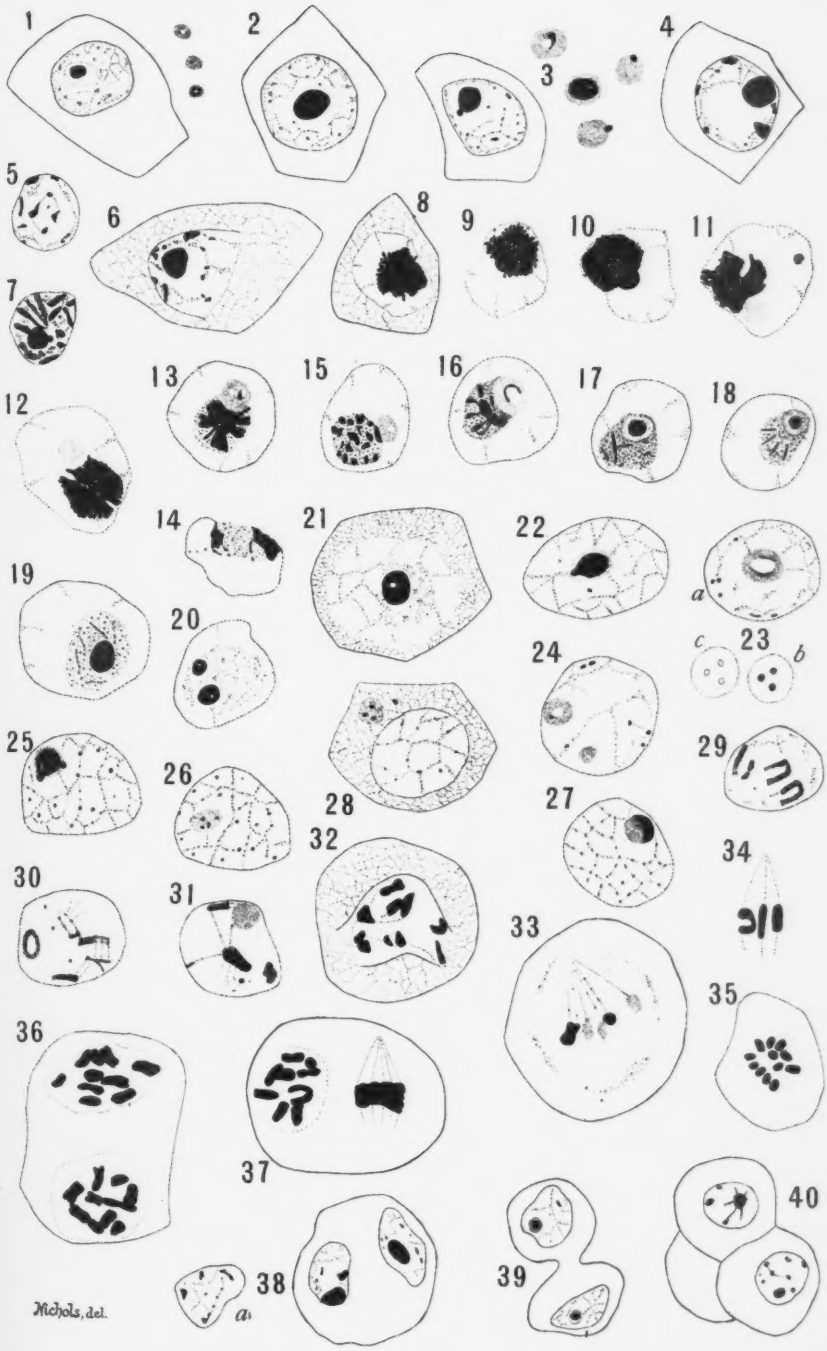

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EXPLANATION OF PLATE V

The drawings were made with the camera lucida, ocular 6, oil immersion objective $\frac{1}{12}$, enlarged to twice the original size and reduced one-half.

Figs. 1–10, S. purpurea; 11, 12, 27, and *34, S. flava; 13–21, 23, 24, 26, 28, S. flava×S. purpurea; 22, 25, 29–32, 33, 35–40, S. variolaris*

FIG. 1. Resting pollen mother cell; *a, b, c,* nucleoli.

FIGS. 2–8. Transition to synapsis; *3a, b, c, d,* nucleoli; *fig. 5,* pole view; *fig. 6,* side view of approximately the same stage.

FIGS. 9–12. Synapsis; gradual emergence of the nucleolus.

FIGS. 13–19. Absorption of chromatin by the nucleolus; *fig. 14,* section below the level of the nucleolus.

FIG. 20. Extension of the achromatic substance.

FIGS. 21–28. Formation of the chromatic network; *figs. 23b* and *c,* nucleoli.

FIGS. 29–32. Prophases of the first maturation division.

FIGS. 33, 34. First maturation spindles.

FIG. 35. Pole view of the same.

FIGS. 36, 37. Second maturation division.

FIGS. 38–40. Completion of the cell divisions and formation of cell walls.

EMBRYOGENY OF ARISAEMA TRIPHYLLUM

JAMES ELLIS GOW

(WITH TWENTY-FOUR FIGURES)

This species is interesting as a representative of the lower monocotyledons, and as an extreme type of its family. If, as suggested by ENGLER, those aroids which possess hermaphrodite flowers are to be taken as the primitive type of the family, and the monoecious genera are to be considered as reduced forms, Arisaema must be regarded as an example of extreme reduction, since it is usually dioecious. According to this view, it should stand farthest in point of development from the original prototype of the aroid family, and although it does not show as complex a structure as do other genera, it should be considered as having probably undergone a longer course of development than they. But it seems quite as reasonable to suppose that the monoecious genera are the more primitive forms, and if this view be accepted, Arisaema must take its place at the foot of the list, as the least differentiated form. If the former hypothesis be accepted, we must regard the occasional monoecism of this species as a case of reversion; if the latter, it must be regarded as a variation which is strictly in accord with the general tendency of the family. It is plain, however, that the problem cannot be solved by merely using the monoecism or dioecism of the species as a basis for classification, but that its whole life-history must be taken into account; and even then the question cannot be answered satisfactorily until the life-histories of all the aroid genera have been worked out with far greater completeness than has yet been done.

The material here described was all gathered in the vicinity of Iowa City, Iowa, during the summer of 1901. A part of it was worked up during the following winter, but it was found necessary to drop the work, and the later phases were not undertaken until the present year. All ovaries were cut out one by one, or in groups of four or five, killed and fixed in 1 per cent. acetic acid, sectioned in paraffin to a thickness of 2–5 μ, and stained with DELAFIELD'S hematoxylin, HAIDENHAIN'S

iron-alum-hematoxylin, or safranin and gentian-violet. In the latter case a saturated solution of each stain in anilin water was used, and very excellent results were obtained.

MEGASPORANGIUM

The ovary of Arisaema contains one to six erect, orthotropous ovules (*fig.* 1). The ovules in over three hundred ovaries were counted, and the average number was found to be four (*fig.* 2). They

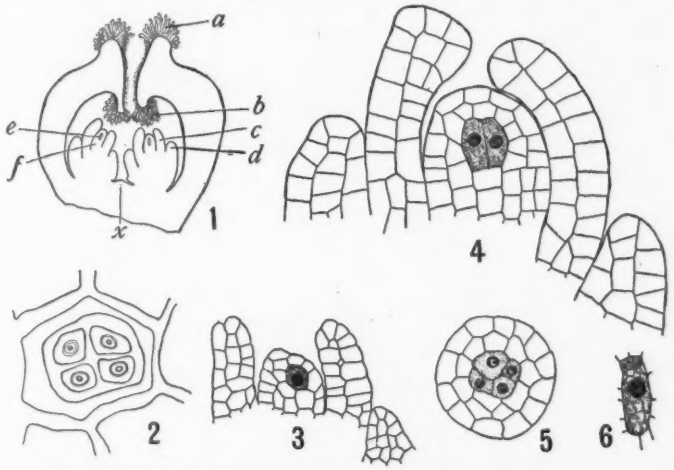
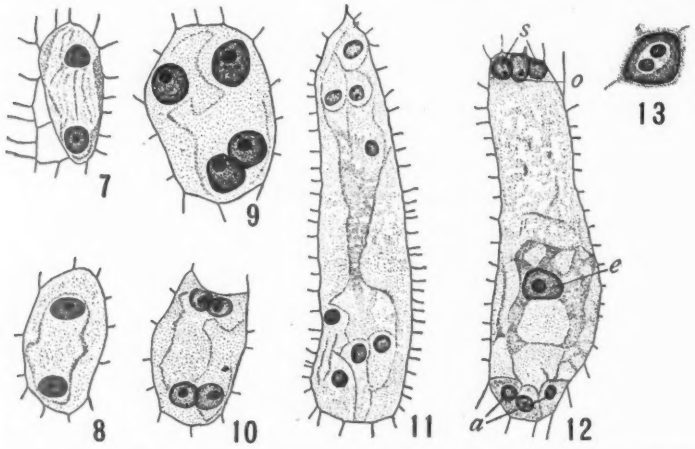


FIG. 1. Ovary of Arisaema: *a*, stigma; *b*, conducting cells; *c*, inner integument; *d*, outer integument; *e*, embryo sac; *f*, nucellus; *x*, end of axis. ×36.—FIG. 2. Transverse section of ovary. ×36.—FIG. 3. Archesporial cell; contents of surrounding cells not drawn. ×385.—FIG. 4. Two sporogenous cells resulting from vertical division of archesporium. ×400.—FIG. 5. Cross-section of nucellus showing four sporogenous cells. ×400.—FIG. 6. Division of sporogenous cell. ×400.

are probably lateral in origin, although at first glance they appear to be axial. The occurrence of four ovules grouped equilaterally about a slight protuberance in the middle of the base of the ovary (*fig.* 1, *x*) would indicate the latter as the suppressed apex of the axis. This reminds one somewhat of the suppression of the axis in Dieffenbachia,[1] except that in Arisaema the suppression is much more complete.

[1] CAMPBELL, D. H., Studies on the Araceae. Annals of Botany 14:1–25. *pl.* 1. 1900.

The earliest material was collected during the first week in April. At that time the ovules appear as minute protuberances from the base of the ovary, the inner integuments have just begun to show, and the cells of the nucellus are as yet undifferentiated. The ovule grows with great rapidity, the outer integument soon appears, and the inner integument passes beyond the apex of the nucellus. At the same time the nucellus itself increases greatly in size, and the



FIGS. 7, 8. First nuclear division of embryo sac. ×920.—FIGS. 9, 10. Second division in embryo sac. ×920.—FIG. 11. The eight-nucleate embryo sac. ×920.—FIG. 12. Embryo sac after fusion of polar nuclei; index letters suggest the structures. ×920.—FIG. 13. Binucleolate endosperm nucleus. ×920.

archesporial cell, recognizable by its greater size and more definite reaction to stains, makes its appearance in the hypodermal layer (*fig. 3*). This is followed by a periclinal division of the epidermal cells of the nucellus. The first two divisions of the archesporial cell are by anticlinal walls at right angles to each other, thus giving rise to four sporogenous cells lying side by side (*figs. 4, 5*). One of these outstrips the others in its growth, crowding them and the surrounding cells of the nucellus. It undergoes one transverse division, by which a small cell, frequently with an ill-defined nucleus, is cut off (*fig. 6*). Whether this is a tapetal cell, or one of a "row of two" formed by a mother cell, was not determined. At this stage the

inner (functioning) cell is usually 0.1^{mm} in length, and its nucleus, by successive divisions, gives rise to the usual eight nuclei of the embryo sac (*figs. 7–11*). The oosphere, synergids, and antipodal cells are organized as usual, and the polar nuclei fuse (*fig. 12*). The fusion endosperm nucleus frequently contains two nucleoli (*fig. 13*).

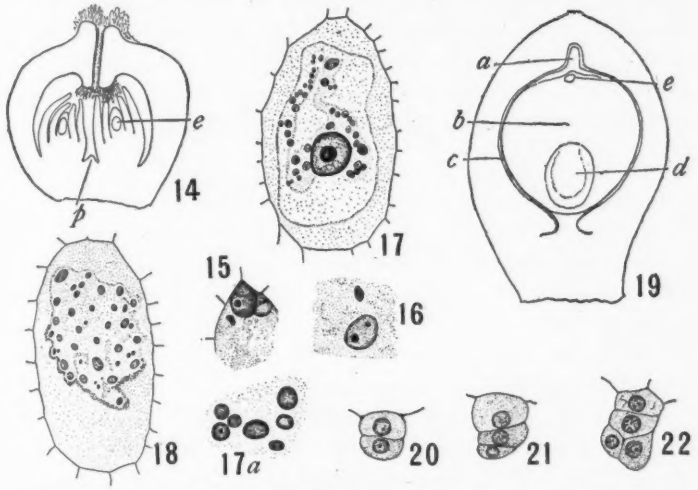


FIG. 14. Ovary at period of fertilization; *e*, embryo sac; *p*, placenta. ×36.—FIG. 15. Oosphere and male nucleus. ×920.—FIG. 16. Endosperm nucleus and second male nucleus. ×920.—FIG. 17. Diagonal section of embryo sac near chalazal extremity, showing endosperm and, probably, one antipodal dislodged by knife. ×920.—FIG. 17*a*. Endosperm nuclei enlarged. ×2050.—FIG. 18. Endosperm nuclei. ×920.—FIG. 19. Longitudinal section of endosperm (*b*), surrounded by remains of inner integument (*c*), and of nucellus (*a*), and containing embryo (*e*), and large vacuole (*d*). ×14.—FIG. 20. Two-celled embryo. ×920.—FIG. 21. Three-celled embryo. ×920.—FIG. 22. Four-celled embryo. ×920.

When the nuclear divisions are complete, the embryo sac has increased to a length of about 0.35^{mm}. The lateral cells of the nucellus have been greatly crowded, but its tip remains intact. The inner and outer integuments have grown until they press against the conducting cells in the roof of the ovary (*fig. 14*).

FERTILIZATION

The stigma of Arisaema consists of a mass of loosely compacted, somewhat mucilaginous papillae (*fig. 11*). The pollen tube finds

access to the interior of the ovary through an open style which is more or less filled with a loose mass of conducting cells, the lower ones extending well down into the cavity of the ovary (*fig. 14*.) The pollen tube enters by the micropyle, and a number of sections were obtained showing it penetrating the nucellus. One section showed the male nucleus and oosphere (*fig. 15*), but careful search failed to bring to light any case of the fusion of the second male nucleus with the endosperm nucleus. As it is known, however, that this "second" male nucleus enters the embryo sac and approaches the endosperm nucleus (*fig. 16*), it seems probable that the fusion actually takes place.

ENDOSPERM

The first divisions from the endosperm nucleus result in the formation of a number of free nuclei (*fig. 17*). These increase in number by successive divisions until they line the interior of the embryo sac (*fig. 18*). They are well defined and usually multinucleolate, showing an extreme diversity in size. When division has proceeded so far as to produce nuclei sufficient to line completely the inner surface of the embryo sac, walls begin to appear, and soon each nucleus is separated from its neighbors by a well-defined wall. Active growth continues until the embryo sac is filled with endosperm, which now begins to crowd the surrounding cells. The lateral vestiges of the nucellus disappear, as a result of this pressure, and the inner integument is stretched until it becomes finally nothing more than a thin limiting membrane surrounding the endosperm. The outer integument remains intact, its growth keeping pace with the growth of the endosperm. The persistent tip of the nucellus likewise remains intact, and even increases in size. The cells in the base of the nucellus are greatly crowded, and many of them break down. When the endosperm has reached its full size, it is about 3^{mm} in length, and consists of two well-defined parts (*fig. 19*). The upper part (*b*) consists of cells with thin but well-defined walls and at first a distinct nucleus; later they are filled with starch grains. In the lower part of the endosperm there is developed a large vacuole (*d*).

EMBRYO

In the specimens examined, the first division of the oospore does not occur until the formation of endosperm is well under way. As

it appears that endosperm formation does not begin until after the pollen tube has reached the embryo sac (and probably not until the endosperm nucleus and the second male nucleus have fused), it would seem that the fertilization of the oosphere is followed by a period of rest before the formation of the embryo begins. The first two divisions are transverse, followed by a longitudinal division (*figs. 20–22*). Beyond this point no definite history of the embryo can be

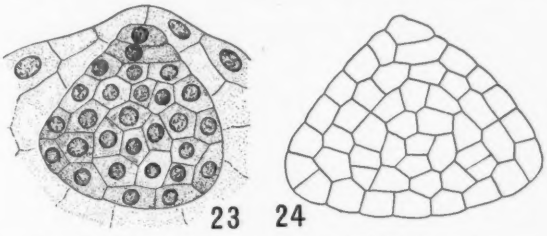


FIG. 23. Longitudinal section of embryo imbedded in endosperm. ×770.—FIG. 24. Same, at somewhat later stage of development. ×770.

given until the stage illustrated in *fig. 23* is reached. The embryo here shown is six weeks or two months old and has not yet reached its maximum development; *fig. 24* shows a somewhat older embryo. Beyond this stage no good sections of the embryo were obtained.

SUMMARY

Although the dioecious character of Arisaema would indicate for it a very low place among the aroid genera, other characteristics do not entirely bear out this view. The erect and orthotropous ovule, it is true, seems to indicate it as a primitive form, but the fact that there are four ovules (typically) in each ovary, and that their basal origin is apparent only, since they are in reality lateral outgrowths of the suppressed axis, seems to prove it a more highly specialized type than Aglaonema, Nephthytis, and Spathicarpa, in all of which each carpel contains a single basal ovule. This view is confirmed if we consider the variations in the number and arrangement of the nuclei in Nephthytis and Aglaonema (CAMPBELL, *l. c.*) as indicating their primitive character. The development of the embryo sac in Arisaema corresponds to the usual type found in other angiosperms, and gives no hint of its possible affinities with lower forms. On the

other hand, the early presence of a number of sporogenous cells in the nucellus appears to ally it with the two genera mentioned, both of which exhibit a similar peculiarity.

The development of endosperm in the aroids is usually characterized from the beginning by the formation of prominent cell walls. This characteristic has been noted by CAMPBELL (*l. c.*) in Anthurium, *Nephthytis liberica*, and Lysichiton, and by myself in *Nephthytis Gravenreuthii*. Arisaema is an exception in that the first formation of endosperm is by free nuclear division, although cell walls are soon developed. In this respect it resembles *Dieffenbachia daraquiniana*, whose embryo sac becomes quite filled with endosperm nuclei before the cell walls begin to make their appearance. The resemblance of the suppressed axis to that of Dieffenbachia has already been mentioned. The anatropous character of the ovule in the latter genus, the fact that each of the two ovules is inclosed in a separate carpel, and the fact that there is only one archesporial cell, and that the primary parietal cell originates at its inner instead of its outer end, serve to draw a broad distinction between it and Arisaema, in spite of the resemblance in endosperm formation. The peculiarities mentioned would probably indicate that Dieffenbachia is a somewhat less primitive form than Arisaema, and one whose development has proceeded along different lines. The presence of staminodia among the pistillate flowers also appears to bear out this view.

Arisaema, therefore, appears to be a more highly developed form than Spathicarpa, Aglaonema, and Nephthytis, but somewhat more primitive than Dieffenbachia, and much more primitive than Anthurium, Spathyema, Orontium, and Acorus, since all the latter have perfect flowers.

When the foregoing was written, the writer was not aware of the existence of a paper by MOTTIER[2] on the development of the embryo sac in Arisaema. A comparison of the two papers, however, shows that they do not cover the same ground, since MOTTIER limited his investigation to the earlier phases, and did not discuss the development of the endosperm, the growth of the embryo, or the possible affinities of the species.

GREENFIELD, IOWA

[2] MOTTIER, D. M., On the development of the embryo sac of *Arisaema triphyllum*. BOT. GAZETTE 17:258–260. *pl. 18*. 1892.

THE TOXIC EFFECT OF CERTAIN COMMON SALTS OF THE SOIL ON PLANTS

FLORENCE N. MAGOWAN

(WITH ONE FIGURE)

A number of investigations have been made upon the action of single salts on plants, but in the majority of cases the object has been to determine the limit of endurance or the highest concentration at which the plants could live in the pure salt solutions. These concentrations vary with the salts used, and from the tabulated results it is not possible to tell the difference in growth of plants in the various salts in equimolecular solutions, nor would it be safe to assume this difference to vary uniformly at all concentrations. On a-priori grounds the reverse might be thought to be true, i. e., comparative degree of toxicity of certain solutions at one concentration might be entirely different at another. Again I have found time to be a very important factor in changing this relation, so that experiments lasting only a short period cannot be considered conclusive. The work done by previous investigators[1] differs so greatly in method, and the results are so scattered that no useful comparison with my results is possible.

Wheat was chosen as a well-known agricultural crop, and one with which much experimental work has been done. One variety only was used, the "Early Genesee," which contains a large proportion of starch, and which in preliminary experiments was found to germinate evenly and with a high percentage of germination. Radish and clover seeds were also used for comparison and found to give similar results to wheat.

In practically all experiments that have been made along this line, the seeds to be tested were first germinated in distilled water, then

[1] References given at the close of the following papers include most of the literature on this subject: "Toxic limits and stimulating effects of some salts and poisons on wheat," by G. H. JENSEN in BOT. GAZETTE 43:42. 1907; also "The variability of wheat varieties in resistance to toxic salts," by L. L. HARTER, U. S. Dept. Agr., Bur. Pl. Industry, Bull. 79. 1905.

placed in the solutions. As the object of this investigation was to find the growth made by the seeds in the different solutions, this method was not practicable, and the seeds were supported on filter paper[2] so that they might germinate in the solution from the beginning.

Twenty or more seeds were placed in each solution. In general the germination was quite uniform. Usually seven duplicate series of experiments were made. While some series made a better growth than others, the comparative order of toxicity was not changed. In all concentrations final examinations were made when the roots had reached the limit of growth. The solutions were all titrated against standard silver nitrate solution in the beginning and great care was taken in making up the various concentrations.

To keep the solutions at the same strength was a problem which received careful consideration. As little salt was taken by the plants the first few days, it was found perfectly safe to replenish the loss due to evaporation by adding distilled water. When the solutions were found by titration to be too weak, however, they were replaced by fresh solutions.

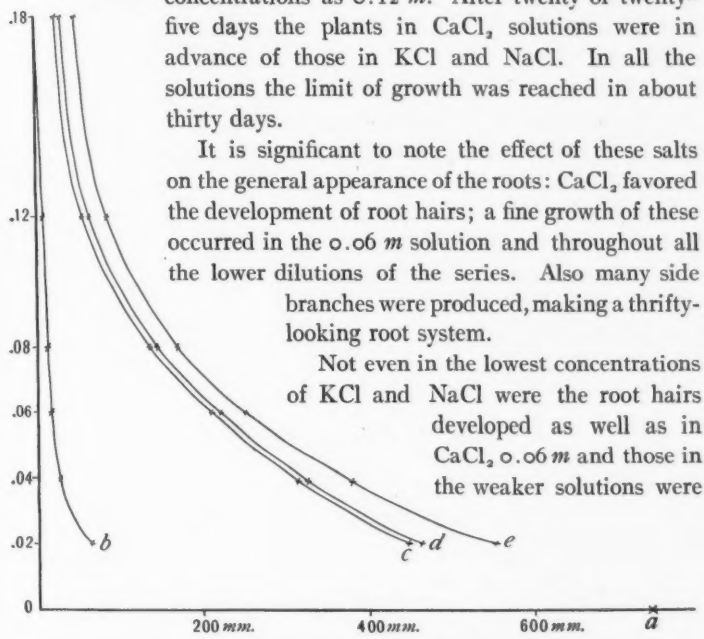
Several concentrations of chlorids of Ca, K, Na, and Mg were used, these salts being taken because they represent the bases most common in the soil. In order that the effects of the cathions might be clearly evident, only one anion, chlorion, was used. Control experiments with tap and distilled water were always carried on with each series, the water having been distilled with especial care to avoid all traces of metals in solution.

The plants made a better growth in distilled water than in any of the salt solutions, which were thus shown to exert a toxic rather than a stimulating effect at the concentrations used. While a marked difference in the degree of toxicity of Ca, Mg, K, and Na was clearly noticeable in the roots, the tops were more nearly alike in equimolecular solutions of the salts, except in the stronger concentrations of $MgCl_2$, in which little growth of either roots or tops occurred.

The order of germination was noteworthy. The seeds in KCl and NaCl were the first to send out roots, and for several days remained in advance of those in $CaCl_2$ and $MgCl_2$. For the first five days the

[2] The method is described by OSTERHOUT, BOT. GAZETTE 44: 259. 1907.

wheat in the $MgCl_2$ solutions made a growth almost equal to those in $CaCl_2$, but after fifteen days the roots in the $MgCl_2$ were dead, while those in the $CaCl_2$ series were still living, even in such high concentrations as 0.12 *m*. After twenty or twenty-five days the plants in $CaCl_2$ solutions were in advance of those in KCl and NaCl. In all the solutions the limit of growth was reached in about thirty days.



It is significant to note the effect of these salts on the general appearance of the roots: $CaCl_2$ favored the development of root hairs; a fine growth of these occurred in the 0.06 *m* solution and throughout all the lower dilutions of the series. Also many side branches were produced, making a thrifty-looking root system.

Not even in the lowest concentrations of KCl and NaCl were the root hairs developed as well as in $CaCl_2$ 0.06 *m* and those in the weaker solutions were

FIG. 1.—Curves showing the growth of roots of wheat in salt solutions; *a*, distilled water; *b*, $MgCl_2$; *c*, NaCl; *d*, KCl; *e*, $CaCl_2$. The ordinates represent concentrations (parts molecular); the abscissae, the aggregate length of roots per plant (in millimeters).

decidedly unthrifty in appearance. In KCl the roots had a dull white color, and as to branching were next to $CaCl_2$, although the branches were thicker and shorter than in $CaCl_2$; the branches were most abundant at 0.02, 0.04, and 0.06 *m*. In $CaCl_2$ branching was practically the same throughout. In NaCl a few stunted branches developed and after twenty days the roots were of a yellow color in all the higher concentrations, down to 0.02 *m*. In $MgCl_2$ neither branches nor root hairs were produced in concentrations 0.08 *m* to

0.005 *m*, but in 0.001 *m* root hairs were developed, and in 0.0001 *m* branches were sent out similar to those in $CaCl_2$ solutions.

The following table gives the average length of roots per plant produced in the various solutions, the figures being based on seven duplicate series of experiments.

Salts	Concentrations					
	0.02 *m*	0.04 *m*	0.06 *m*	0.08 *m*	0.12 *m*	0.18 *m*
$MgCl_2$	64mm	26mm	15mm	10mm	6mm	0mm
NaCl	446	321	210	134	54	24
KCl	462	333	220	144	63	31
$CaCl_2$	552	386	249	168	84	48

Distilled water, 740mm

These results are also shown in *fig. 1* in the form of curves, the concentrations being used as ordinates and the average length of the roots per plant being used as abscissae. The curves show that little growth took place at 0.18 *m* in sodium, potassium, and calcium chlorids, and none whatever in magnesium chlorid. The small growth in magnesium chlorid throughout the whole series of concentrations is due mostly to its toxic action; but also in some degree to its higher osmotic pressure. The molecule of $MgCl_2$ when dissolved dissociates into three ions instead of two, as in the case of NaCl and KCl; hence $MgCl_2$ solutions have a higher osmotic pressure than equimolecular solutions of NaCl and KCl.

All the more striking is the behavior of $CaCl_2$ which in spite of high osmotic pressure (approximately as high as $MgCl_2$) nevertheless permits greater growth than NaCl or KCl; hence it must be much less toxic than any of the other salts.

RESULTS

1. Each of the principal soil bases (Na, K, Mg, Ca) is toxic. The following is the order of toxicity of their chlorids: (1) $MgCl_2$, (2) NaCl, (3) KCl, and (4) $CaCl_2$.

2. Experiments lasting but a short time (such as have been made by most previous investigators of the toxic action of salts) cannot be considered as conclusive as longer experiments. After six days,

plants in NaCl and KCl made more growth than in $CaCl_2$, but after twenty-five or thirty days the plants in $CaCl_2$ were in advance of all the others.

These investigations were carried on under the direction of Professor W. J. V. OSTERHOUT, to whom the writer wishes to express sincere thanks for his kindly assistance and valuable suggestions.

UNIVERSITY OF CALIFORNIA
Berkeley, Cal.

BRIEFER ARTICLES

THE PHYSIOLOGICAL CONSTANTS OF PLANTS COMMONLY USED IN AMERICAN BOTANICAL LABORATORIES

The object of this paper is identical with that of its predecessor,[1] namely, to ascertain which of the plants commonly grown or readily raised in greenhouses is best adapted for the demonstration of a particular physiological process, and how much may be expected of it and of the others. The work is being done in the Laboratory of Plant Physiology at Smith College, with the advice of Professor W. F. GANONG. Others of the series are in advanced preparation.

II. ROOT PRESSURE AND EXUDATION

Osmotic processes, of course, are of the greatest importance in the life of the plant, and of these osmotic root absorption is perhaps best known. The most direct way of demonstrating the osmotic absorption and pressure of the root is by measurement of the quantity and pressure of exudation from the stem cut just above the ground. I have undertaken to find for those greenhouse plants which are practicable for the purpose the pressure of root exudation and the quantity exuded. The only work of importance done upon potted plants, so far as I can find, is that of WIELER[2] in 1892, who recorded the pressures of some fifteen greenhouse plants, and this is the source of the figures in current books. Single measurements have also been made by DETMAR and a few others. The pressures given by trees lie outside the present work.

The first requisite for the study of exudation pressure is a gauge which will register the pressure accurately, however small the quantity of liquid exuded. Most of those used for demonstration are faulty in principle to an extreme degree, as has been recently pointed out.[3] After some preliminary trials, I have fixed upon one which is practically that described by MORSE and FRASER[4] for the measurement of osmotic pressures. It is a mercury manometer of the usual form, of 0.5^{mm} bore barometer tubing, with a bulb blown in the middle arm. In order to test the variations in

[1] BOT. GAZETTE **40**:302–305. 1905.

[2] Cohn's Beiträge zur Biologie der Pflanzen **6**:1–210. 1892.

[3] W. F. GANONG in School Science and Mathematics **6**:300. 1906.

[4] Am. Chem. Jour. **34**:3–5. 1905.

the diameter of the bore, two gages were calibrated. It was found, however, that while for high pressures, involving the measurement of short lengths of tube, it would be necessary to make the correction for the differences in diameter of the bore, this is not needed in dealing with small pressures, of about one atmosphere, because in the length of tube used the inequalities, which are very slight, balance one another.

For use the gage is first filled with clean, dry mercury; then with the lower end dipping into boiled water mercury is forced out into the water by pressure with a rubber bulb upon the upper end until there remains only the required amount, namely, enough to fill the gage half-way up the bulb. When the force is removed, water replaces the mercury in the lower end. The long arm is then filled with air, dried, by being drawn through tubes containing calcium chlorid and phosphorus pentoxid, and is quickly sealed with hot, melted shellac. Proper tests were of course applied to prove that the shellac made a perfectly tight joint with the glass. Sealing wax was used at first, but it is apt to crack from the glass when the range of temperature is great. The gage was then attached by means of stout-walled rubber tubing to the stump of a plant whose top had been cut off $2\text{–}3^{cm}$ above the ground. The cutting was done under water to prevent the entrance of air, which would rise in the gage later. The rubber tubing was fastened on the stump with stretched rubber bands, or wire in case of a stout stem, and to the manometer by wire and was wound with tire-tape to prevent stretching under internal pressure.[5]

Where the stem was larger than the gage, a glass sleeve was cemented with sealing-wax outside the latter, to make it nearly the size of the stem. The height of the mercury in the long arm was noted and when it had reached its highest point the pressure was computed by BOYLE's law. A source of error in the use of the gage, not noted until too late for correction, consists in the expansion of the mercury through rise in temperature. Subsequent tests were made to ascertain its extreme possible amount, with a result that under no circumstances could the error exceed 0.0134 atmospheres or 0.2^{lb} per square inch, and in nearly all cases it was very much less than this.

The quantity of exudation was found at the same time for plants of the same age and approximate size as those used in the exudation pressure experiments. For this a piece of glass tubing was attached to the plant and led into a graduated cylinder which contained a film of oil to prevent evaporation.

[5] A fuller description of this gage with directions for use is given by W. F. GANONG in a catalogue of apparatus now being published by the Bausch & Lomb Optical Company.

All the plants used were young, in good growing condition, and in most cases were taken just before flowering. It will at once occur to the reader that in some cases conclusions have been drawn from too small a number of plants, but since in these experiments the plants must be sacrificed completely, it was impossible to obtain more than three of some species—one for exudation and two for pressure. However, this does not involve so great a possibility of error as it seems at first sight, for, although it is possible that the plant used may be abnormal, the probabilities, according to QUETELET'S law, are that it will fall near the mean. The temperature of the laboratory during observation of the experiments was 18°–22° C. The soil temperature at time of highest pressure was 17°–18°C.

The results obtained for quantity and pressure of exudation are given in the following table, in which the first and fourth columns give the number of plants used for exudation and for pressure experiments, respectively.

From the table on page 53 it will be seen that the plants giving the highest mean pressures, in order of amount, are *Salvia involucrata*, *Helianthus annuus*, *Fuchsia speciosa*, with others following according to the table. When considered in order of maximum pressures, the highest are given by *Fuchsia speciosa*, *Salvia involucrata*, and *Lycopersicum esculentum*. The highest minimum pressures are given by *Helianthus annuus*, *Salvia involucrata*, and *Senecio Petasitis*.

For the practical purpose of demonstration, taking into consideration high pressures, ease of manipulation, and abundance of plants, the very best are *Fuchsia speciosa*, *Chrysanthemum frutescens*, and *Pelargonium zonale*. Since they have stout, woody stems, there is no danger of compressing the vessels in making a tight joint with the pressure gauge. With the pressure gauge attached they maintain a high pressure for about a week, usually giving the highest pressure between 10 and 12 o'clock on the second day. The next best are *Heliotropium peruvianum*, *Pelargonium peltatum*, and Abutilon. These give lower pressures than the preceding, but they have a slight advantage in another way. The stems of vigorous young plants have about the same diameter as the manometer tubing and therefore can be more readily attached to the gauge. Other plants which give high pressures are *Lycopersicum esculentum* and *Phaseolus vulgaris*. These have somewhat soft stems, though with care it is not difficult to attach the gauge.

The following plants cannot be used advantageously for this experiment: *Euphorbia pulcherrima*, because of its latex which thickens and closes the ducts in a very short time; *Ricinus communis*, because of its hollow stem; *Senecio mikanioides* and *Tropaeolum majus*, because their

Name	No. of plants	Exudation quantity in cc.	Durat'n of flow in days	No. of plants	Pressure in atmospheres
		Min. Mean Max.			Min. Mean Max.
Abutilon (golden bells)...	3	1 – 1.9–3.3	2–3	4	.757– .795– .840
Begonia coccinea.....	1	168	29	2	.818– .858– .898
Chrysanthemum frutescens (marguerite).....	4	24 –40 – 55	9–21	4	.847–1.014–1.230
Coleus.....	2	11.5–14.2– 17	8–9	2	.822– .897– .972
Cucurbita Pepo (squash)	1	27	12		
Euphorbia pulcherrima (poinsettia).....	1	2.5	1	2	.573– .637– .701
Ficus elastica (rubber plant).....	1	23	31	2	.737– .817– .897
Fuchsia speciosa.....	6	16.5–99.2–263	12–34	8	.887–1.246–1.605
Hedera Helix (English ivy).....	1	1.5	2	2	.323– .430– .538
Helianthus annuus (sunflower).....	1	30	16	2	1.210–1.276–1.343
Heliotropium peruvianum (heliotrope).....	1	7	8	4	.809–1.045–1.294
Impatiens Holstii.....	1	3	3	2	.851– .855 .860
Lupinus albus (white lupine).....	1	4	3	2	.793– .807– .821
Lycopersicum esculentum (tomato-dwarf stone).....	1	13	5	4	1.015–1.164–1.349
Pelargonium domesticum (Lady Washington geranium).....	1	17.4	13	2	.714– .861– .909
Pelargonium peltatum (ivy-leaved geranium)	1	4.5	9	2	.940–1.001–1.063
Pelargonium zonale (horse-shoe geranium)	3	14–15.5–17.5	19–20	10	.620– .881–1.116
Phaseolus vulgaris (string bean).....	2	4– 4.2– 4.5	5	4	.908–1.002–1.149
Ricinus communis (castor bean).....	1	8.5	17	2	.666– .692– .718
Salvia involucrata.....	1	14	12	2	1.151–1.369–1.571
Senecio mikanioides (German ivy).....	1	27	7	2	.518– .633– .648
Senecio Petasitis.....	1	110	14	2	1.137–1.191–1.246
Tropaeolum majus (nasturtium).....	1	13.5	8	2	.759– .779– .800
Zea Mais.....	1	4	6		

stems are too soft and weak. I could not attach the gauge successfully to the following: *Cucurbita Pepo*, *Zea Mais*, and *Vicia Faba*. Obviously stemless plants cannot be used. Thus comparatively few of the common plants are practicable.

The minimum pressures for any given plant in the table were obtained in the winter months and the maximum pressures in April and May, showing a variation in pressure with time of year which is probably widespread.

In order to determine the individual variations of plants taken at the same time of year and treated in exactly the same way, the following species were studied with appended results:

April, 5 plants Pelargonium zonale, .620, .645, .767, .782, 1.035 atmospheres.
June, 5 plants Fuchsia speciosa, 1.406, 1.428, 1.467, 1.500, 1.605 "

While an average of the pressures given by the above plants may seem of no great value, nevertheless there is a certain advantage in knowing this mean, which may be taken as a general expression of the root pressure of common greenhouse plants. The mean pressure of the 22 plants studied is approximately 0.9 atmosphere, or in round numbers 13^{lb} for the square inch.

Turning from pressure to quantity of exudation, the above table shows that the plants giving the greatest quantity of exudation are *Fuchsia speciosa* and *Begonia coccinea*. Different plants of *Fuchsia speciosa* vary greatly in the quantity given off, and this seems to be correlated with the formation of new shoots—the longer this is delayed the greater the flow. This is true in less degree of most of the plants which form new shoots. In others—*Impatiens Holstii*, *Lycopersicum esculentum*, *Pelargonium peltatum*, and *Tropaeolum majus*—the stems decay and the roots soon die.

Some observations were made on periodicity of exudation and of exudation pressure. These facts will be published in a separate note later.—SOPHIA ECKERSON, *Northampton, Mass.*

THE CONDITION OF CERTAIN WINTER BUDS

During 1905–1906 an effort was made to ascertain the seasonal stages in the microspore development of certain woody plants, with the following results:

	Mother cell stage	Dividing nuclei	Mature spores
Populus deltoides	.....	.....	October 23
Fraxinus americana	Oct. 23—Feb. 15	.....	.....
Celtis occidentalis	October 24	April 12	April 15
Carpinus caroliniana	October 24	April 12	April 15
Cornus florida	August 20	August 22	August 24
Cercis canadensis	October 31	April 17	April 20

WILLIAM MOORE and MARY E. BEHNEY, *Ursinus College, Pa.*

CURRENT LITERATURE

BOOK REVIEWS

Danish strand vegetation

WARMING[1] has published the first of a series of important monographs on the vegetation of Denmark. The aim is to treat the entire vegetation of the country from the standpoint of ecology. In view of the many years of work of the author in this field, these monographs are certain to take front rank in studies of this kind, and it is to be regretted that publication in the Danish language makes them inaccessible to many who would read them with keen interest. The first monograph deals with the strand, and the fact that more than 300 pages are devoted to the strand of a small area, and at that without including the sand-dune vegetation, gives some conception of the wealth of detail and thoroughness of treatment of the study. In WARMING's many years of work in Denmark, the strand and dune vegetation of his country have especially attracted his interest, and the work here reviewed may be regarded as a summary of all his previous work on the strand. There is no doubt that it takes its place at once as the classic contribution on this subject. The volume is amply illustrated with many halftone reproductions from photographs and a large number of drawings, representing life-habits and structural characteristics of the strand plants.

The first chapter considers the sea-cliffs, a physiographic formation that is but slightly developed in Denmark. The granites of Bornholm are inhabited by halophytic lichens, and the Cretaceous calcareous rocks furnish a habitat for rock-boring algae. Chapters follow on the gravel and shingle shores. There are all gradations from rough stony coasts with no vegetation to older and higher gravel coasts with almost closed vegetation carpets. Between large stones there often appear the characteristic plants of salt marshes. The most widespread shore formation is the sand strand, and here successive belts of vegetation are seen, first a plantless zone, then a zone of sand algae, followed by a zone of halophytic seed-plants, mostly chenopods; last of the truly maritime formations is a zone in which various non-halophytic psammophytes mingle with the halophytes. A chapter is devoted to the vegetation of the seaweed heaps, mostly made up of ruderal chenopods. The shallow lagoons, known as "vader," are poor in plants but rich in animal life (for a fuller account of these interesting habitats, see BOT. GAZETTE **41**: 78, 79. 1906); sometimes various diatoms and *Salicornia herbacea* are the only plants of such places. In similar situations, where only spring tides bring contact with salt water, are some remarkable alga societies, chiefly com-

[1] WARMING, EUG., Dansk Plantevaekst. 1. Strandvegetation. 8vo. pp. vi + 325. *figs. 154.* Copenhagen and Christiania. 1906.

posed of Chlamydomonas, Pleurococcus, various blue-green algae, Myxophyceae, and diatoms. The animals of the "vader" and these algae are of enormous importance in reclaiming land from the sea. A chapter is devoted to the enalids, that is, the plants of submerged meadows, among which Zostera, Ruppia, and similar plants dominate.

An entire chapter is devoted to the ecology of *Salicornia herbacea* (another evidence of the thorough treatment of the book), which is the pioneer emergent plant of the salt marsh; its upper line is determined by the tide, since over three hours of complete emergence seems detrimental. Beyond the Salicornia zone are various halophytic plants, largely grasses, whose distribution varies largely with soil changes. Sand-binding grasses like *Glyceria maritima* dominate in the sandy marshes of this type. In the chapter which compares the salt marshes of the North and East seas, it is noted that the North Sea flora is much more halophytic than is that of the less salty East Sea. It is very curious, however, that some true halophytes of the East Sea region are not known from the North Sea. Reed swamps of Phragmites, Scirpus, etc., are found in brackish waters (up to 3 per cent. salt). Of much interest are the bacterial swamps in which the purple sulfur bacteria and Beggiatoa dominate; these occur of course where there is decaying vegetation. A chapter is devoted to the unevennesses of salt marshes (such as mounds formed by ants, moles, etc.), and their vegetation, which differs much from the ordinary flora of the marsh. The influence of artificial land reclamation is the subject of another chapter; dike-building soon results in the disappearance of salt from the marshes, and the development of the vegetation into an artificial meadow. The final chapters deal with the ecological characteristics of halophytes. Most species are hapaxanthic turf-builders. Woody plants are missing except for two half-shrubs. Succulence and leaf isolaterality are common. The flowers are largely wind-pollinated, and the seeds are scattered more by water currents than by other means. The thoroughness of this work makes us long for the other members of the series.—H. C. COWLES.

A new textbook of biology

That the teaching of biology in secondary schools has not yet reached an accepted method is evident with the appearance of each new textbook. Formerly courses in general biology were recommended, without any separation into botany and zoology. Later it became more common to offer unit courses in zoology, or botany, or physiology. During all this time, however, there have been adherents to the elementary course in general biology, and some excellent arguments for such a course have been recognized by almost every teacher.

In a recent book by HUNTER[2] we have another attempt to solve this problem. It is intended to present botany, zoology, and human physiology in one course to students in the first year of the high school, and has been used in the New

[2] HUNTER, GEORGE WILLIAM, Elements of biology. pp. 445. New York: American Book Company. 1907.

York city schools. The sequence is botany (146 pp.), zoology (139 pp.), and human physiology (124 pp.). Preceding the presentation of botany, there is an introductory chapter, another on "Experiments in chemistry and physics," and a third on "Protoplasm and the cell." Evidently there is no attempt to follow the older type of courses in general biology, but rather to present in one book short courses in the three sciences. Directions for experiments accompany the discussions of the text and are often not dissociated from them. The preface states that "it would not be wise to attempt all of the work outlined in this book;" and also, "it is thought that each successive chapter, although related to that immediately preceding it, is yet distinctive enough to allow of the omission of a chapter or chapters without in any way interfering with the continuity of the work." This universal plasticity suggests a general lack of organic relationship.

The botany begins with a chapter on flowers. The first two pages are almost entirely filled with fine print, which the author says in his preface is "less important;" and with this luminous beginning we find on the third page the astonishing title "Fertilization of flowers," in connection with which appear diagrams and terms presenting ovules, embryo sac, eggs, pollen grains, and pollen. The attitude of mind which makes possible the attempt to present this difficult topic in this way is shown, not only by the misleading title given above, but by the following sentence: "The first beginning of the growth of the seed takes place at the moment of fertilization." The remainder of the chapter discusses pollination; and then follow chapters on "Fruit" and "Seeds and seedlings." It is not until the seventh chapter that there is any discussion of the structure or functions of the working parts of the plants. Even then the chapter on "Roots and their work" is followed by a chapter on "Buds and stems," instead of the chapter on "Leaves and their functions." In such an arrangement no close working relation is developed between the different regions. Ecology is presented in one chapter (8 pp.), and the botany closes with a somewhat long chapter on "Flowerless plants."

If throughout this part of the book there were evident an attempt to correlate the material with that of zoology or physiology, we might understand the plan, but no such correlation appears; in fact there is not even any correlation of the botanical material itself. Furthermore, the statements are often faulty. In defining matter, the author says: "It may be living, or may have been alive at some previous time, in which case we speak of it as organic matter; or it may never have been alive. The latter state of matter is called inorganic." Under the topic "chloroplasts," the process of photosynthesis is discussed, and in italics the statement is made that "the chloroplasts, by means of the energy received from the sun, manufacture starch out of certain materials." An accompanying diagram, labeled "Diagram to illustrate the formation of starch," attempts to visualize the process; but not a word appears to indicate that starch is not formed directly by chloroplasts, or that anything besides starch is made by photosynthesis.

The treatment of zoology is from the standpoint of increasing complexity, from Protozoa to Mammalia. For this subject the author is equipped, so that probably it is free from such faults as have been indicated in the chapters on

botany. The section on human physiology attempts to cover all the important topics, and meets the demands of certain state laws by indicating the effects of alcohol. The absurd extent to which this may be carried is shown in the chapter on "The nervous system," in which ten pages are given to alcoholism and eight to the entire nervous system.—OTIS W. CALDWELL.

Wettstein's Handbuch [3]

A third instalment of WETTSTEIN'S *Handbuch* contains a general discussion of the angiosperms and a special taxonomic treatment of the choripetalous dicotyls. The next and final instalment, which is promised in the spring of 1908, will deal with the rest of the dicotyls and with the monocotyls. After a presentation of the general morphology of angiosperms, the writer discusses their phylogeny. He believes that the monocotyls have been derived from the dicotyls, and that among the dicotyls the Monochlamydeae contain the most primitive forms. Consequently, they would be most likely to show characters which might indicate relationship with groups below. The pollen-tube structures and the flowers of angiosperms are thought to indicate a gymnosperm origin. It is not claimed that the flowers of living gymnosperms and angiosperms furnish an easy transition, but merely that the types can be reconciled both morphologically and ecologically. The Choripetalae are arranged in thirteen series, beginning with the Verticillatae, Casuarinaceae being the lowest family, and ending with the Centrospermae, in which the Caryophyllaceae are the highest family. The Dialypetalae contain twelve series, beginning with the Polycarpicae, Magnoliaceae being the lowest family, and ending with the Umbelliflorae. The description of families is full and clear and profusely illustrated with excellent figures, many of which are new.—CHARLES J. CHAMBERLAIN.

MINOR NOTICES

Das Pflanzenreich.[4]—Parrt 30 contains the Styracaceae, prepared by JANET PERKINS. After the usual introductory account of the characters and geographical distribution of the family, the six genera are presented as follows: Pamphilia (3 spp., 1 new), Styrax (97 spp., 11 new), Bruinsmia (2 spp.), Alniphyllum (3 spp.), Halesia (3 spp.), and Pterostyrax (3 spp., 1 new).

Part 31 contains the Potamogetonaceae by ASCHERSON and GRAEBNER, prefaced by an unusually full account of the structure and habits of this interesting family. The systematic presentation is as follows: Zostereae containing Zostera

[3] WETTSTEIN, DR. RICHARD R. v., Handbuch der systematischen Botanik. II Band. 2 Theil (erste Hälfte). pp. 161–394. *figs. 165* (995). Leipzig und Wien: Franz Deuticke. 1907. *M* 9.

[4] ENGLER, A., Das Pflanzenreich. Heft 30. Styracaceae von J. PERKINS. pp. 111. *figs. 18* (191). *M* 5.60. Heft 31. Potamogetonaceae von P. ASCHERSON und P. GRAEBNER. pp. 184. *figs. 36* (221). *M* 9.20. Leipzig: Wilhelm Engelmann. 1907.

(6 spp.) and Phyllospadix (3 spp.); Posidonieae containing Posidonia (2 spp.); Potamogetoneae containing Potamogeton (89 spp., 1 new, and numerous hybrids) and Ruppia (1 sp.); Cymodoceae containing Cymodocea (7 spp.) and Diplanthera (2 spp.); and Zannichellieae containing Zannichellia (2 spp., 1 new) and Althenia (4 spp.).—J. M. C.

Giesenhagen's Lehrbuch.—The fourth edition of GIESENHAGEN'S *Textbook of botany*[5] is not essentially different from the third edition, which was reviewed in this journal.[6] A few details have been modified, a few figures added, and the text in general brought up to date. The most noticeable change is the presentation of current theories, which many botanists believe to be out of place in a textbook. The author, however, believes that important theories and problems, even though still lacking definite proof, are valuable if the presentation makes it clear that they are only theories.—CHARLES J. CHAMBERLAIN.

Plants of Bolivia.—The fourth part of the enumeration of Bolivian plants collected by MIGUEL BANG has been issued as a Bulletin of the New York Botanical Garden.[7] The previous parts appeared in the *Memoirs of the Torrey Botanical Club* in 1893, 1895, and 1896. The present part completes the enumeration with the exception of Gramineae, and contains descriptions of 227 new species. Three new genera are also characterized: *Tournefortiopsis* and *Poederiopsis* (both Rubiaceae), and *Vassobia* (Solanaceae).—J. M. C.

Genera Siphonogamarum.—The eleventh fascicle of DALLA TORRE and HARMS'S[8] list of the genera of seed-plants completes the work, the last entry being Zyzygium. There are included also title-page, preface, and list of families (290 in number).—J. M. C.

NOTES FOR STUDENTS

Morphology of bryophytes.—Several studies of bryophytes come to hand almost simultaneously. DACHNOWSKI[9] has been working again upon the much-studied *Marchantia polymorpha*. The growth of the rhizoids, the origin of dorsiventrality, the plagiotropic position, the formation of reproductive organs, and fertilization have been reexamined. His conclusions are in part like those of previous investigators and in part a slight modification of them. He finds moisture the most

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[5] GIESENHAGEN, DR. K., Lehrbuch der Botanik. Fourth edition with 561 illustrations. 8vo. pp. xiv+463. *figs. 561*. Stuttgart: Fr. Grub. 1907.

[6] BOT. GAZETTE **37**: 225. 1904.

[7] RUSBY, HENRY H., An enumeration of the plants collected in Bolivia by MIGUEL BANG. Part 4. Bull. N. Y. Bot. Garden **4**: 309–479. 1907.

[8] DALLA TORRE, C. G. DE, and HARMS, H., Genera Siphonogamarum ad systema Englerianum conscripta. Fasc. 11. pp. 801–921. Leipzig: Wilhelm Engelmann. 1907. *M* 6.50.

[9] DACHNOWSKI, A., Zur Kenntnis der Entwicklungs-Physiologie von *Marchantia polymorpha*. Jahrb. Wiss. Bot. **44**: 254–286. *figs. 4. pl. 4*. 1907.

important factor in calling forth rhizoids. Dorsiventrality is fixed in 10–20 hours after sowing and "rests upon the reciprocal relation between determinative cooperating external factors and internal conditions of maturity"—the latter dependent in large measure upon the age of the gemmae. The plagiotropic position is a functional relation, influenced by illumination, and resulting from the cooperation of diaheliotropism and negative geotropism. Moisture and other variable external factors exercise a far greater influence than diageotropism, autogenous hyponasty, and photepinasty, to which it has been previously referred. As to reproductive structures, they seem to arise only when the plants are brightly illuminated, whereas weak illumination and much moisture permit only vegetative growth. Natural parthenogenesis was not observed.

GYÖRFFY has had opportunity to study that curious hybrid, *Physcomitrella Hampei*, which has three forms, differing according to the male parent, *Physcomitrella patens* being in all cases the female parent. One of these is *P. patens* × *Physcomitrium sphaericum;* the second, *P. patens* × *Physcomitrium eurystomum;* and the third *P. patens* × *Physcomitrium pyriforme*, which GYÖRFFY himself discovered. He has made a comparative anatomical study of the first and third hybrid forms and of their parents. This is a paper that will be useful for the writer of a comparative anatomy of mosses, but for readers it is wordy and tiresomely detailed, and might easily have been compressed, to its advantage, into half the space. It seems hardly necessary in such a paper to describe the form of an archegonium and the mode by which the canal is opened for the sperm, when these do not have any peculiarities; the paper is full of such repetitious and irrelevant matter. This begins in the title, which is an excellent example of what a title ought *not* to be.[10] GYÖRFFY makes one particularly interesting observation, which is important if true; he figures the spore mother cells as capping the columella in *P. patens*. This is contrary to LIMPRICHT's observations, and as GYÖRFFY lays no special stress on the point, it seems doubtful if he appreciated its significance. In general he finds, as others have done, that the vegetative characters are those of *P. patens*, while the characters of the sporophyte are intermediate in certain respects. Thus the foot and the external form of the sporophyte of *P. Hampei* I are those of *P. sphaericum*, while the internal structure is more like that of *P. patens*. In *P. Hampei* III, strangely enough, the form of the capsule is like that of *P. Hampei* I, though the foot and long seat are those of *P. pyriforme*.

SERVIT discusses the mode of branching of the bryophytes,[11] supporting the novel views of VELENOVSKÝ,[12] which take no account of the source of branch

[10] GYÖRFFY, I., Ueber die vergleichenden anatomischen Verhältnisse von *Physcomitrella patens* (Hedw.) Br. et Sch., *Physcomitrium pyriforme* (L.) Brid., *Physcomitrium sphaericum* (Ludw.) Brid., und *Physcomitrella Hampei* Limpr. [*Physcomitrella patens* (Hedw.) × *Physcomitrium sphaericum* (Ludw.), resp.: *Physcomitrella patens* (Hedw.) × *Physcomitrium pyriforme* (L.)]. Hedwigia **47**: 1–59. 1907.

[11] SERVIT, M., Ueber die Verzweigungsart der Muscineen. Beihefte Bot. Cent. **22**[1]: 287–293. *figs. 3.* 1907.

[12] VELENOVSKÝ, J., Vergleichende Morphologie **1**: 130. Prag. 1905.

initials, and determine morphological relationships merely by "how things look in the mature condition."

SERVIT extends VELENOVSKÝ's "angular leaf" idea from pteridophytes to bryophytes, and describes such a leaf in Mastigobryum, where it is easily distinguished by its transverse insertion as well as by its form. It is situated at the base of two branches and he considers it as formed by the concrescence of the first leaf of both. This angular leaf, he says, is present in all liverworts whose branches arise from the half-segment; but it is elsewhere not so typical (this sounds Goethean) as in Mastigobryum. It is lacking in those whose branches arise only from the basiscopic part of the half-segment.

In intercalary branching both of liverworts and mosses the branch is endogenous and breaks through a sheath, which may be split into segments that SERVIT calls *Blättchen*. He has confirmed in all cases VELENOVSKÝ's "law" (that the branches in mosses arise without exception in the axil of the leaf below them!) by examining "extraordinarily abundant material;" but he does not say that in any case he has determined the relation of the branch initial to the leaf initial. Until that is done the current view, which rests upon careful studies of LEITGEB, will prevail.

BUCH has made a thorough study of the two modes of vegetative propagation of *Blasia pusilla*,[13] especially of the development and germination of the gemmae produced in the flasklike receptacles. These arise both on male and female plants, but are rare on the latter if they form embryos. While the brood-buds (modified tips) are the chief means of propagation in summer, the gemmae probably do not germinate in the summer and certainly not in the autumn, but hibernate and start new plants in the spring. Shoots arise from either side (seldom both), where there is a zone of four small cells. One of the upper two produces a thallus initial by three successive divisions of the protruded tube, while the two lower ones produce rhizoids.—C. R. B.

Mutations of Oenothera.—MACDOUGAL[14] and his collaborators have published a further account of evolutionary studies upon the Oenotheras. Taxonomic descriptions of several of the mutants from *O. Lamarckiana*, as they grew in the New York Botanical Garden, are given, including *O. albida*, *O. oblonga*, *O. scintillans*, *O. brevistylis*, and *O. lata*. These will be valuable for comparison with cultures elsewhere. The English "*O. biennis*," as growing in the vicinity of Liverpool, has been found to be *O. Lamarckiana*, and growing wild with it are two of the mutants, *O. rubrinervis* and *O. lata*, the latter maturing pollen, contrary to its habit elsewhere. The cultures of *O. Lamarckiana* from DEVRIES' seeds gave six of the mutants described by that author and also nine other types which could

[13] BUCH, H., Ueber die ungeschlechtliche Vermehrung von *Blasia pusilla* (Mich). L. Öfvers. Finska Vet. Soc. Förhdl. **49**:no. 16. pp. 42. *pls. 2. figs. 7.* 1907.

[14] MACDOUGAL, D. T., VAIL, A.M. and SHULL, G. H., Mutations, variations, and relationships of the Oenotheras Carnegie Institution of Washington, Publ. no. 81. pp. 92. *pls. 22. figs. 73.* 1907.

not be identified. These aberrant forms are of special interest from the standpoint of variation, and it is hoped that further careful records will be obtained of them and their offspring.

SHULL presents a continuation of his earlier studies[15] on variation in *O. Lamarckiana* and some of its mutants. On account of the unsatisfactory character of height, branching, and leaf form for statistical comparisons, the studies are extended to include bud characters. The fact that all his collections of buds were made late in the season, and that there is a marked seasonal variation in the size of the buds, would render somewhat doubtful his statement that the periodicity is probably too small to need consideration. The bud characters chosen are length and thickness of ovary, hypanthium, and cone, the forms studied being *O. Lamarckiana*, *O. rubrinervis*, *O. gigas*, and *O. lata*. A formidable series of variation and correlation tables is presented, the general conclusion being that the mutants tend to show greater variability than the parent form, though there is no decrease in correlation of parts. MACDOUGAL concludes on these premises that instead of mutations being the cumulative results of ever-increasing fluctuation, they are the initial process, of which fluctuations in the mutants, gradually decreasing to a minimum, are an after-effect. The hypanthium of Oenothera, which is the most variable part of the bud, is considered an example of a phylogenetically new character showing increased variability.

Miss VAIL contributes taxonomic accounts of *O. grandiflora*, *O. Simsiana*, *O. Oakesiana*, *O. parviflora*, and *O. muricata*. *O. parviflora*, known in Europe since 1759, has recently been found native in Maine.

Cultures of *O. grandiflora* and *O. biennis* showed the presence in the former of at least two mutants, and that under the latter there is a swarm of elementary species distributed throughout North America. Two interesting cases of bud-sports were observed, one in *O. Lamarckiana*×(*O. Lamarckiana*×*O. cruciata*), and the other in *O. ammophila*, which is suspected of being a hybrid derivative of *O. biennis*. In both cases self-fertilized seeds of the mutant branch came true to the type of the sport.

A careful account is given of MACDOUGAL'S injection experiments. After some negative results with other forms, mutants were obtained from injected ovaries of *O. biennis* and *Raimannia odorata*. The solutions used were $ZnSO_4$ (1:500), $Ca(NO_3)_2$ (1:1000), and 10 per cent. sugar solution. Other inflorescences were treated with a pencil of radium emanations. In the *O. biennis* experiments, in addition to the usual forms, one rosette appeared which differed widely from any known type, and its progeny was like it. In the case of *Raimannia odorata*, a group of mutants appeared in the progeny of injected ovaries. It was also found that "plants of the progeny of the first treatment which were apparently normal yielded seeds which gave a few atypic forms."

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[15] MACDOUGAL, D. T., assisted by VAIL, A.M., SHULL, G. H., and SMALL, J. K., Mutants and hybrids of the Oenotheras. Carnegie Institution of Washington, Publ. no. 24. pp. 57. *pls. 22. figs. 13.* 1905.

BOULENGER,[16] from observations of the evening primroses growing wild at South Kensington, England, and in La Garde St. Cast, Brittany, concludes that *O. Lamarckiana* and *O. biennis* cannot be distinguished as separate "species," and that *O. Lamarckiana* originated from *O. biennis* through some hypothetical process of hybridization among the different forms of the latter. His conclusions rest on an examination of the variability of the plants taken *en masse* in the wild condition, and the unproven assumption that all the plants observed at St. Cast, Brittany, came from a single stock. Many different forms, both in Europe and America, go under the name of *O. biennis*, as MACDOUGAL has shown, and if *O. Lamarckiana* were capable of giving rise to any of these we might have expected to find them in DEVRIES' cultures. The study of the Oenotheras has long since reached the stage where cultures are necessary to determine the relationships of the various strains, and without such studies speculations drawn from casual observations of variability among forms growing wild are not likely to affect seriously the results obtained from culture work such as that of DEVRIES.—R. R. GATES.

A biometric study of Ceratophyllum.—A recent memoir by PEARL[17] on variation and differentiation in Ceratophyllum presents an admirable illustration of the successful application of mathematics to the working-out of important biological problems. The work is too compact to allow a brief review to present all the important relations which are clearly demonstrated by biometric analysis of the variation in the number of leaves in a whorl as related to the position of the whorl on the plant. It is shown that the whorls on each grade of branches, e. g., main stem, primary, secondary, tertiary, etc., are differentiated as a class from each other grade by several distinctive features of their variability. In all these grades the correlation between number of leaves in a whorl and its position on the stem is considerable. It is least in the main stem and increases as we pass to the more peripheral divisions. The regression line is not linear, but logarithmic, being of the type $y = A + C\log(x - a)$, in which A, C, and a are constants, y is the number of leaves per whorl, and x the position of the whorl. The curves so derived fit beautifully the observed data, and allow the derivation of what is believed to be a very fundamental biological law which is called the "first law of growth" in Ceratophyllum, and is stated thus: "On any axial division of the plant the mean number of leaves per whorl increases with each successive whorl in such a way that both the absolute increment and the rate of increase diminish as the distance (measured by the number of nodes) of the whorl from a fixed point increases."

By the fact that the equation fits data from plants collected from different habitats, by merely changing the factor A which represents the actual size and

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[16] BOULENGER, G. A., On the variations of the evening primrose (*Oenothera biennis* L.). Jour. Botany **45**:353–363. 1907.

[17] PEARL, RAYMOND, assisted by OLIVE M. PEPPER and FLORENCE J. HAGLE. Variation and differentiation in Ceratophyllum. Carnegie Institution of Washington, Publ. no. 58. pp. 136. *pls. 2. figs. 26.* 1907.

not at all the size of parts relative to position, it is shown: (1) that A is the environmental factor, and (2) that environment affects size but not at all the progress of differentiation represented by the logarithmic curve. It is found that the curve for the main stem is most nearly horizontal and that for each successive grade of branches is steeper and steeper, a change in the curve dependent upon the increasing value of the constant C, which is therefore regarded as the constant which expresses the action of internal formative factors.

On any axial division the first whorls are the most variable in number of leaves, and this variability decreases at first rapidly, then less and less rapidly as more and more distal positions are reached, the theoretical limit attained being perfect constancy. This constitutes the "second law of growth" in Ceratophyllum. It is shown that these two laws are true in branch production as well as leaf production in this species.

Although few other studies have been made which can be definitely cited to demonstrate the general validity of these two laws of growth, the essence of the second law was recognized by the reviewer[18] in the case of *Sium cicutaefolium*, and other published observations are mentioned which appear to indicate that these are general laws. It is believed that this second law is the same which in the physiological field has been stated by JENNINGS[19] thus: "An action performed or a physiological state reached is performed or reached more rapidly after one or more repetitions, so that in time it becomes 'habitual.'" It is shown that in psychology it is probably the same law that lies at the basis of memory, namely, the ease, as measured by rapidity and accuracy, with which a state once reached is reached again. It seems probable, at any rate, that suitable analysis of all these cases will show that they are governed by logarithmic laws, and though no suggestion has been made as to the nature of the common causes upon which such general validity of the logarithmic law must rest, their nature will doubtless be brought to light very soon when once it is recognized that they exist.

The necessity of more studies of a similar nature is apparent. It is important to know how these laws are affected by more complex environmental conditions. The ease and conclusiveness with which they were demonstrated in Ceratophyllum probably depended upon the fact that in this species environmental modifications are reduced to their lowest terms. It is to be hoped that the aims and methods of this paper may serve as guides for many biometric studies, for the successful analysis of the laws of differentiation within the individual must precede studies which involve repeated structures in different individuals. Studies of the latter type are insecure until the laws of intra-individual differentiation and variation are known for the particular species of plant or animal under investigation.—GEO. H. SHULL.

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[18] SHULL, G. H., Stages in the development of *Sium cicutaefolium*. Carnegie Institution of Washington, Publ. no. 30. pp. 28. *pls.* 7. 1905.

[19] JENNINGS, H. S., The methods of regulation in behavior and other fields. Jour. Exp. Zool. 2:473–494. 1905.

Taxonomic notes.—C. WARNSTORF (Hedwigia **47**:76–112. 1907), in describing new species of Sphagnum, includes 5 from the United States and 9 from S. America.—ED. FISCHER (Ber. Deutsch. Bot. Gesells. **25**:372–376. 1907) describes in a preliminary way some hypogeous fungi from California, collected by SETCHELL and GARDNER, including a new monotypic genus, *Pseudobalsamia Setchellii*, and a new species of Pseudogenea and of Hysterangium. He also refers Pseudhydnotrya to Geopora (Pezizaceae).—R. C. BENEDICT (Bull. Torr. Bot. Club **34**:445–458. 1907), in a first paper on the genus Antrophytum, has presented a synopsis of the subgenera and the American species, the latter 9 in number, 2 of which are new.—F. D. KERN (*idem* 459–463) has published new western species of Gymnosporangium (3) and Roestelia (3).—W. A. MURRILL (*idem* 465–481), in a list of Philippine Polyporaceae, has published 24 new species and transferred 21.—J. N. ROSE (Smithson. Miscell. Coll. **50**:330. 1907) has published a new Nopalea from Guatemala; and in collaboration with N. L. BRITTON (*idem* 331–333. *pls. 43, 44*) has established a new genus (*Pereskiopsis*) of Cactaceae, by raising to generic rank § Pereskiopuntia of Opuntia, including 11 species, 2 of which are described as new.—L. M. UNDERWOOD and W. R. MAXON (*idem* 335, 336) have described 2 new species of Lindsaea, one from Columbia, the other from Cuba.—W. F. WIGHT (*idem* 174. *pl. 17*) has described a new Alaskan larch (*L. alaskensis*).—C. V. PIPER (*idem* 195–202), in publishing some new plants of the Pacific slope, describes 3 new subspecies of *Cassiope Mertensiana;* presents a revision of *Orthocarpus tenuifolius* and its relatives, including a new species; and describes new species under Lupinus, Castilleia, and Valeriana.—F. S. COLLINS (Rhodora **9**:197–202. *pl. 76.* 1907) has described new species of green algae under Pleurococcus, Chaetomorpha, Cladophora, and Vaucheria (2).—A. DAVIDSON (Muhlenbergia **3**:105–108. 1907), in a discussion of several species of Sphaerostigma, describes a new species.—In the first paper (Bull. Soc. Bot. France **54**:1–30. 1907) of a series to contain descriptions of new plants collected in tropical Africa by AUG. CHEVALIER, CASIMIR DECANDOLLE describes a new genus (*Charia*) of Meliaceae.—In the 19th number of "Diagnoses Africanae" (Kew Bull. 1907:360–365), W. B. HEMSLEY has described a new genus (*Cordeauxia*) of Leguminosae (Caesalpinieae), and H. BOLUS a new genus (*Peglera*) of Rhizophoraceae.—N. L. BRITTON (Bull. N. Y. Bot. Gard. **5**:311–318. 1907), in a fourth contribution to the flora of the Bahama Islands, describes new species under Zamia, Ibidium, Badiera, Passiflora, Rochefortia, and Tetranthus.—G. W. WILSON (Bull. Torr. Bot. Club **34**:387–416. 1907), in a second paper on N. Am. Peronosporales, presents the Phytophthoreae and Rhysotheceae, and describes *Rhysotheca* as a new genus of 12 species, to include Peronospora § Zoosporatoparae DeBary.—P. A. RYDBERG (*idem* 417–437), in his 18th paper on the Rocky Mountain flora, breaks up Thelypodium, as heretofore recognized, into 6 genera, the 5 new genera being *Thelypodiopsis, Pleurophragma, Hesperidanthus, Stanleyella,* and *Heterothrix;* establishes a new genus (*Chlorocrambe*) on *Caulanthus hastatus* S. Wats.; and describes new species under Homalobus (5), Diholcos, Kentrophyta, Aragal-

lus (3), Hedysarum, Lathyrus, Capnoides (2), Lepidium (3), Thelypodium (2), Pleurophragma, Sophia (2), and Arabis.—J. M. C.

Ammonifying soil nitrogen.—LIPMAN has made another valuable contribution to soil bacteriology.[20] His experiments were exceedingly well planned, were fundamental, and in most cases gave consistent results. So much detail is included in the report that a satisfactory summary of the article is impossible.

He first tested the ability of various soils to ammonify nutrient solutions of peptone, and solutions containing peptone and inorganic salts. The addition of the salts favors the growth of certain species of bacteria and also ammonification under certain conditions. These species were unequally distributed in soils. Inoculation with soil infusion was decidedly inferior to inoculation with soil itself. But soil infusions in the same sterile soil exhibit relations similar to those of the corresponding direct soil inoculations. The inoculation of sterile soils in various ways shows that in ammonification the bacteriological soil factor is much more important than the chemical one. Manured soils showed a superior ammonifying power. Peptone salt solutions which are not provided with too large a quantity of mineral are most suitable for study of the general condition of the bacterial flora of the soil. The differences in the power of ammonification exhibited by several soils seem to bear no direct relation to their mechanical composition. The amount of the sterile soil which is inoculated affects the yield of ammonia, the amount of ammonia being larger, in nearly every case, in the larger samples of soil. This may be due to increased production of ammonia by ammonification of the soil nitrogen; or, more probably, to the larger amount of other mineral substances present which may be useful to the organism; or possibly to the absorption of deleterious products by the soils. Old samples of soil kept in a laboratory for some months had decidedly lower ammonifying power than the younger samples. Original differences in the ammonifying power of the different soils, however, were not entirely obliterated, even after keeping the samples in the laboratory for several months.

Lime added to the soil often stimulated the activity of decay bacteria, the stimulus increasing with the amount of lime up to two tons per acre. The author suggests that it may be possible to develop a bacteriological method of determining the lime requirements of soils in general. More consistent results were obtained by soluble nitrogenous material, such as peptone and gelatin, than by insoluble substances, like albumen, casein, and cotton-seed meal. As a rule, ammonia is produced very rapidly; the amounts given off in three days were nearly as large as in thirty-two days, and in some cases they were larger.

Working with Azotobacter, LIPMAN found that small quantities of soil did not furnish enough mineral salts, whereas in larger quantities a normal growth was obtained. He therefore suggests that it may be possible to measure the mineral

[20] LIPMAN, G. B., Chemical and bacteriological factors in the ammonification of soil nitrogen. Report N. J. Agric. Exp. Sta. **1906**: 119–187. 1907.

deficiencies of soils by determining how much soil is required to give normal Azotobacter formation.—F. L. STEVENS.

Fossil Osmundaceae.—KIDSTON and GWYNNE-VAUGHAN[21] describe the structure of two osmundaceous stems from the Jurassic of New Zealand. On account of the difficulty of distinguishing anatomically between Osmunda and Todea, they put these stems under the provisional genus *Osmundites*. One of the stems, *O. Dunlopi*, is characterized by an extremely small central cylinder, comparable with that found in the filmy Todeas of the present day. In this species the foliar gaps are much reduced and are said by the authors even to be absent sometimes. The other new species, *O. gibbiana*, has a better developed stele and its foliar gaps are very similar to those of living species of Osmunda. The authors add something to our knowledge of the *Osmundites skidegatensis* of PENHALLOW, from the Lower Cretaceous of Western Canada. In this species they describe well-developed internal phloem. The medullary tissues clearly become continuous with those of the cortex through the very broad foliar gaps. Concerning this species the authors say: "At first sight (it) appears to provide something very like the dictyostelic ancestor postulated by JEFFREY'S theory." They conclude, however, with BOODLE and the majority of other British anatomists, since there is an absence of internal phloem in the young plant of living species of Osmunda, that on the hypothesis of recapitulation internal phloem and the accompanying structures must have been absent in the ancestors of the Osmundaceae. This argument, however, is quite fallacious. It would apparently be just as reasonable to assume that the mesarch foliar and peduncular bundles of the Cycadales are not an ancestral feature, as is generally admitted, especially in Great Britain, because forsooth they have not been shown to occur in the stem of cycadean seedlings. Moreover, those who hold with GWYNNE-VAUGHAN, WORSDELL, and others, that the condition without internal phloem is ancestral in the case of the tubular central cylinder of the stem in the Filicales, find likewise no ontogenetic support whatever for their views among the very numerous ferns in which the tubular central cylinder of the adult is siphonostelic with internal phloem. Ontogeny at present throws no light whatever on the subject of the presence or absence of internal phloem in the primitive tubular central cylinder of the fern series. Argument accordingly must be based on comparative anatomy alone, and that being the case there is apparently as good reason to consider the concentric type of bundle to be ancestral for the leaf and stem of the Osmundaceae as there is to regard the mesarch xylem bundle as ancestral for leaf and shoot in the cycadean series. The authors follow SCOTT in regarding the Botryopterideae as among the most primitive ferns. They consider that their *Osmundites Dunlopi* furnishes evidence that the Osmundaceae have come from this group. There is apparently no better reason for regarding *Osmundites Dunlopi* and *O. gibbiana* as primitive Osmundaceae than the similarly austral types, Agathis and Araucaria, as primitive araucarian conifers.—E. C. JEFFREY.

[21] KIDSTON, R., and GWYNNE-VAUGHAN, D. T., On the fossil Osmundaceae. Trans. Roy. Soc. Edinburgh **45**:759–780. *pls. 1–6.* 1907.

Ophioglossaceae.—CAMPBELL[22] has published an account of his studies of East Indian species of Ophioglossum. He collected *O. moluccanum*, *O. intermedium*, and *O. pendulum*, securing both gametophytes and sporophytes in considerable number. The spores of *O. moluccanum* germinated freely, but owing to a failure to become associated with the mycorhizal fungus the prothallia died after reaching the four-celled stage. Prothallia of *O. pendulum* made the mycorhizal connection and reached the thirteen-celled stage. They did not develop any chlorophyll on exposure to light, although a few chloroplasts developed in the prothallia of *O. moluccanum*. The adult gametophyte in all the species studied is subterranean, without chlorophyll, and radiate in structure. CAMPBELL'S account agrees closely in this regard with the accounts of METTENIUS, LANG, and BRUCHMANN. The account of the development of the antheridium is also essentially the same as that given by these authors. The statement is made that in the mitosis immediately preceding the formation of sperms the number of chromosomes is much larger than in the earlier divisions. Most cytologists will regret that this interesting situation was not investigated somewhat further. The development of the sperm agrees closely with that of Equisetum. The archegonia resemble those of the Marattiaceae. There is either a binucleate neck canal cell or two neck canal cells. The ventral canal cell is difficult to demonstrate, but was shown to be present in *O. pendulum* and probably in the others. The primary wall in the embryo is transverse in most cases, but varies considerably, particularly in *O. pendulum*. The entire hypobasal half goes to the formation of the foot. There are three types of embryo: (1) that of *O. moluccanum*, in which only root and leaf are developed, the plant being annual and no stem being produced; (2) that of *O. vulgatum*, in which root and stem are first produced, and much later a leaf; and (3) that of *O. pendulum*, in which root only is produced, stem and leaf arising later by means of an endogenously developed bud from the primary root at some distance from the gametophyte; the vascular bundles of leaf and stem arise separately from the bundle of the root and remain separate.

In *O. moluccanum* the primary root is monarch and the bundle of the leaf is collateral, and CAMPBELL says that it is merely a question of terms whether one calls the root monarch or collateral. The primary root of *O. pendulum* is diarch, as are also the later roots. The *O. moluccanum* type of embryo is probably the most primitive and is most nearly related to the embryos of Marattiaceae and Equisetineae, especially to the former. The author's well-known claim that Ophioglossum is a primitive type of pteridophytes is emphasized by his somewhat detailed explanation as to how the *O. moluccanum* type might have arisen from an Anthoceros-like ancestry.—L. LANCE BURLINGAME.

Sterility in hybrids.—TISCHLER[23] has published a preliminary account of further investigation on the causes of sterility in hybrids. He has studied *Potentilla*

[22] CAMPBELL, D. H., Studies of the Ophioglossaceae. Ann. Jard. Bot. Buitenzorg II. **6**:138–194. *pls. 9–19*. 1907.

[23] TISCHLER, G., Weitere Untersuchungen über Sterilitätsursachen bei Bastardpflanzen. Ber. Deutsch. Bot. Gesells. **25**:376–383. 1907.

Tabernaemontani×*P. rubens*, *Syringa vulgaris*×*S. persica*, and Mirabilis forms including *M. Jalapa*×*M. tubiflora* and *M. Jalapa*×*M. longiflora*. In some of these the usual irregularities in the reduction mitoses of sterile hybrids are reported. In *Potentilla Tabernaemontani*×*P. rubens* the x number of chromosomes is 16. The hybrid shows no difference from the parent *P. Tabernaemontani* in pollen formation, except that the former produces a larger number of imperfect pollen grains. In *P. rubens* nearly all the pollen grains are good. The latter is a constant species, while the former is very variable or "mutable." By subjecting this hybrid to etiolation in hothouse temperature, complete sterility was produced. The reduction divisions were apparently normal, but there was a lack of cytoplasm and of chromatin, the scarcity of the former beginning as early as the archesporium stage. Similar but less marked results were obtained by subjecting *P. rubens* to the same conditions. Some of TISCHLER'S interesting conclusions, which will be further discussed in the forthcoming paper, may be briefly stated as follows: (1) Sterility of hybrids does not depend upon any form of chromatin repulsion. (2) Sterility results from the fact that two sexual cells are thrown together whose developmental tendencies are not identical. When the individual reaches the critical reproductive stage the disturbance of harmony in its development makes itself apparent. (3) By modification of the external conditions in plants which are not hybrids, the sexual cells may be so influenced as to produce conditions similar to those which result from hybridization. (4) The sterility of hybrids is a purely relative matter. (5) A true splitting or segregation of characters does not occur in the reduction divisions. One of the arguments in support of this conclusion is that certain characters Mendelize which concern not single rudiments but the constitution of the whole of the idioplasm. (6) The assumption that the individual characters are connected with distinct and separated pangens is abandoned. (7) The chromatin is not of exclusive significance as a hereditary substance. (8) There is a specific idioplasm having a definite constitution. (9) A distinction is to be maintained between chromatin and linin. (10) Apogamy is merely an aid in reproduction in cases of pollen sterility, and is not the primary condition to which pollen obliteration is secondary. (11) Similarly, as shown by CORRENS, in species which are passing from the monoecious to the dioecious condition, disturbances similar to those of mutation occur, resulting in contabescence of the anthers or ovaries. (12) There is, as DARWIN believed, a close relation between the sterility of hybrids and that of plants under cultivation. The full account of this work will be awaited with interest.—R. R. GATES.

Anatomy of Araliaceae.—A very comprehensive treatment of the Araliaceae has been published by VIGUIER,[24] dealing with the history of the family, the external morphological characters, and the internal structure of stem and leaf of about sixty genera. Besides being a valuable contribution to the knowledge of plant anatomy in general, the paper offers an excellent proof of the importance of ana-

[24] VIGUIER, RENÉ, Recherches anatomiques sur la classification des Araliacées. Ann. Sci. Nat. Bot. IX. 4:1–208. 1906.

tomical characters to systematic research. As a matter of fact, the anatomical characters as described by VIGUIER are not only very useful, but to some extent really necessary for gaining a thorough knowledge of this family and its affinities. From an external morphological point of view the Araliaceae exhibit a number of very conspicuous types, represented by herbaceous, frutescent, and arborescent species; among these the herbaceous are of a special interest, and it is to be regretted that the author could not examine the root structure of the very peculiar Panacineae, for example, to which the ginseng belongs. The stem structure seems to be very constant. The cuticle is often thick; the epidermis consists only of a single layer; the cortex is differentiated as an external continuous zone of collenchyma and an inner parenchymatic zone frequently traversed by secretory ducts; the pericycle is stereomatic and forms arches, always accompanied by secretory ducts; the cork develops constantly in the peripheral hypodermal stratum of cortex, the only exception being Echinopanax. A very considerable variation exists in the occurrence of ducts in the pith, of mestome strands in the pith, in the presence or absence of secretory cells (*poches sécrétrices*) in the secondary hadrome, etc., which is readily noticeable from the anatomical table. The petiole shows also a very characteristic structure, and among the generic characters may be enumerated the arrangement of the mestome in one or several concentric bands; the various disposition of the ducts; the collenchyma continuous or interrupted by cortical, chlorophyll-bearing parenchyma, etc. The structure of the petiole becomes very much complicated when secondary formations set in, as observed in several species. In the blade of the leaf the structure is quite variable, as follows: secretory cells occur only in Gilibertia; the midrib may be composed of several mestome strands arranged in a circle, or may consist only of an arch of leptome and hadrome; hypodermal collenchyma is developed in some genera, but not in others. The anatomical keys are drawn so well that the genera may be easily identified in a number of cases. Ten tribes are recognized by the author, and some new genera have been proposed. Aralidium has been excluded; it lacks secretory ducts and might be placed more naturally as a member of Cornaceae, near Torricellia, Melanophylla, and Kaliphora.—THEO. HOLM.

Embryology of Sechium.—LONGO[25] has found that in the maturing seed of Sechium (Cucurbitaceae) the outer integument enlarges, consisting of homogeneous parenchyma traversed by vascular strands and rich in starch. No "testa" development occurs, but the ovule is closely invested by the pericarp. The outer integument is thus a food-storage region for the embryo, which uses up the endosperm, all of the nucellar tissue, and the inner integument. In germination the cotyledons are differentiated into two regions, intraseminal and extraseminal. The stomata of the two regions differ markedly, in the intraseminal region being more circular in outline and with more circular slits than shown by the decidedly elongated outline of guard cells and slits of the extraseminal region. The differentiation is possibly associated with the differentiation into absorbing and photosynthetic regions.—J. M. C.

[25] LONGO, B., Sul *Sechium edule* Sw. R. Accad. Lincei **16**: 470–472. *figs. 2.* 1907.

Seed dispersal in Polygonum.—REED and SMOOT[26] find that *Polygonum virginianum* has a somewhat unique means of seed dispersal. When an object hits the rigid persistent style, the akene is violently ejected. It is found that a definite separation layer is developed, and that a considerable tension arises in the pedicel through the growth of the pith below the separation layer. This growth is sufficient to cause the compression of the pith above the layer, since the bounding vascular cells are rigid. The release of these cells from this pressure, when the akene is detached by a stroke, causes the shooting of the akenes to a distance of three or four meters.—H. C. COWLES.

Solution tension and lipolysis.—POND[27] has investigated the effect of various toxic salts upon the saponification of ethyl butyrate by a commercial product, holadin. Whereas MATHEWS, MCGUIGAN, and CALDWELL concluded from their results with eggs of Fundulus, diastatic digestion, and proteolysis, respectively, that toxicity was an inverse function of solution tension, POND concludes that in lipolysis this is not true of the salts tested. He points out also various discrepancies in the results of these investigators, emphasizing the want of agreement and the extent of the divergence of observed from calculated values.—C. R. B.

Swiss vegetation.—Few countries are better known phytogeographically than Switzerland. GRISCH[28] has given a detailed account of one of the less known districts. The entire area studied contains only subalpine, alpine, or nival vegetation. Much is made of the influence of snow, and it is pointed out that snow is as detrimental to some plants as it is favorable to others. This was brought out by some interesting experiments that supplemented the field study. A detailed treatment of the various plant formations, and an annotated list of the species make up the body of the paper.—H. C. COWLES.

Heliotropism in a lichen.—NĚMEC has tested experimentally an inference of SACHS from observations in nature that the thallus of *Peltigera canina* reacted to light like the thallus of Marchantia.[29] He finds *Peltigera aphthosa* distinctly heliotropic, without having the rate of this reaction influenced by geotropism, and with a non-reversible inherent dorsiventrality. The young rhizines are negatively heliotropic. The perceptive and active regions have not been determined.—C. R. B.

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[26] REED, H. S., and SMOOT, I., The mechanism of seed-dispersal in *Polygonum virginianum*. Bull. Torr. Bot. Club **33**:377–386. 1906.

[27] POND, R. H., Solution tension and toxicity in lipolysis. Am. Jour. Phys. **19**: 258–283. 1907.

[28] GRISCH, ANDR., Beiträge zur Kenntniss der pflanzengeographischen Verhältnisse der Bergünerstöcke. Beih. Bot. Centralbl. **22**²:255–316. 1907.

[29] NĚMEC, B., Die heliotropische Orientation des Thallus von *Peltigera aphthosa* (L.) Hoffm. Bull. Int. Acad. Sci. Bohême **11**:1–5. 1906.

NEWS

DR. PERCY GROOM has been appointed lecturer in botany at the North London Polytechnic.

DR. C. DETTO of Leipzig, known for his treatise on the theory of adaptation and for other ecological studies, is dead.

MR. OAKES AMES, North Easton, Mass., has presented his collection of living orchids to the New York Botanical Garden.

DR. N. L. BRITTON, N. Y. Botanical Garden, has been elected an honorary member of the Philadelphia College of Pharmacy.

DR. A. A. LAWSON (Chicago), recently of Stanford University, has been appointed assistant in botany at Glasgow University, to succeed Mr. GWYNNE-VAUGHN.

HARVARD UNIVERSITY received in June last a bequest of $50,000 to be used in connection with the Gray Herbarium. It is known as the Sarah E. Potter endowment fund.

MR. R. P. GREGORY has been appointed lecturer in botany at Cambridge University, to succeed Mr. A. W. HILL, who has become assistant director of the Royal Garden at Kew.

PROFESSOR WILLIAM BATESON, Cambridge University, during his recent visit to the United States, lectured at the Brooklyn Institute of Arts and Sciences, and at The University of Chicago.

DR. HANS FITTING, *privat-docent* at the University of Tübingen, has received for 1907 the stipend of the German government which provides for a visit to the Buitenzorg (Java) botanic garden.

DR. J. REYNOLDS GREEN, lately professor of botany to the Pharmaceutical Society of Great Britain, has been appointed to the Hartley lectureship on plant physiology at The University of Liverpool.

THE DEATH IS ANNOUNCED of Dr. FRANZ KRAŠAN of Graz, Austria. Dr. KRAŠAN is perhaps best known by his writings concerning evolution, which contain records of interesting experiments, as well as somewhat speculative conclusions. In the field of plant geography, he was interested especially in the influence of the earth's heat as a phytogeographic factor, and also in the vegetation of Styria.

A NEW BOTANICAL JOURNAL has been established at Stockhlom, with the title "Svensk Botanisk Tidskrift." It is published by the Swedish Botanical Society, the editorial committee being O. ROSENBERG, K. BOHLIN, G. LAGERHEIM, N. SVEDELIUS, and R. SERNANDER, with ROSENBERG as editor. Four parts will be issued each year, the subscription price being 15 kroner. Two parts for 1907 have appeared, containing about 300 pages.